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SEROLOGIC CHANGES AFTER ORAL RAGWEED POLLEN THERAPY IN CHILDREN

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THE value of oral pollen therapy has been the subject of numerous conflicting reports in the literature, which have been well summarized recently by Feinberg and his collaborators.[1] These investigators carried out a cooperative study of the merits of oral pollen therapy in several clinics during the summer of 1939 and reached the conclusion that the therapeutic effectiveness of oral pollen therapy is at the highest estimate of a minor grade, particularly in the control of asthma.

It seems that children obtain better results from oral pollen therapy than do adults. In some preliminary work done by us in the summer of 1938, we noted excellent results in eight of ten children treated for uncomplicated fall hay fever by the oral method. The maximum dose reached in our patients was 240,000 Noon pollen units. Bohner[2] reported that in his experience children received more benefit from oral pollen treatment than did adults. It is interesting to note that in the report of Feinberg and his collaborators[1] a group of twelve children ranging in age from 12 to 15 years were studied separately by one (E. P.) of the investigators. The results were strikingly different in this group from the others reported. Nine of the group had fall hay fever. Three had autumnal asthma. Eleven of the twelve received ragweed pollen orally while the twelfth received placebo capsules.

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The maximum doses given are shown in Table I. All the patients in this group, including the patient receiving placebo capsules, had marked to moderate improvement.

Another group of fifteen children studied by two other investigators (B. Z. R. and M. Z.) are presented in the same report. Of the seven who were asthmatic, none showed improvement on oral ragweed pollen therapy. Of the eight who had simple fall hay fever, two were improved and six were not. The dosage given to these patients was 60,000 units in one case and from 120,000 to 180,000 units in the remainder. These dosages are definitely lower than those given to the more successfully treated group of children. The authors are uncertain as to whether this accounted for the difference in the results which they reported or whether it was largely a difference of interpretation by different observers.

TABLE I

NO. OF PATIENTS	DOSE POLLEN UNITS
1	120,000
3	180,000
1	140,000
3	360,000
3	420,000

ABSORPTION OF INGESTED RAGWEED POLLEN

Much of the discussion in the literature in regard to the merits of oral pollen therapy centers about the question of proof of absorption. Thommen[3] and Black[4] demonstrated the presence of ragweed antigen in the blood and urine after the ingestion of large amounts of ragweed pollen. The basis of their conclusion that ragweed pollen was absorbed from the gastrointestinal tract was the observation of positive intracutaneous tests with such blood serum and urine in ragweed-sensitive persons and those previously sensitized. C. Bernstein, Jr., and Kirsner[5] concluded that enteral absorption in four persons did not occur since passively sensitized sites failed to react after the ingestion of a capsule containing 0.3 Gm. (300,000 pollen units) of ragweed pollen. T. B. Bernstein and Feinberg[6] tested enteral absorption in seventeen nonallergic subjects. Doses as high as 5 Gm. (5,000,000 pollen units) were administered. In only one person who had a bleeding carcinoma of the stomach was evidence of absorption obtained by a positive reaction at the sensitized site. Zeller[7] and London[8] confirmed these observations by employing a combination of the methods used by Bernstein and Feinberg, with those of Black, and concluded that definite evidence of enteral absorption was still lacking.

Since none of the absorption studies mentioned above were carried out on children, it was deemed advisable to repeat the experiment of

Bernstein and Feinberg as a preliminary to our subsequent studies. Five children, not sensitive to ragweed and between the ages of 6 and 8 years, were passively sensitized for ragweed with a serum containing a high titer skin-sensitizing antibody, by injecting 0.1 c.c. intracutaneously. The following day each child was given 5 Gm. (5,000,000 pollen units) of ragweed pollen in 1 ounce of water before breakfast. The sensitized sites were observed at intervals for the next twenty-four hours. No reactions occurred. Twenty-four hours after the administration of the ragweed pollen by mouth, 1.0 c.c. of a ragweed extract containing 0.5 mg. of protein nitrogen per cubic centimeter was injected subcutaneously in the anterior aspect of the thigh. Strong reactions occurred at the sensitized sites of all recipients, indicating that sufficient absorption for desensitization of these sites had not occurred from the ingested pollen.

It is difficult, however, to understand how relief can be obtained by oral pollen therapy, unless the pollen or its extract enters the blood stream. Experimental methods have thus far not yielded conclusive evidence of such absorption. The fact remains that clinically, in some pollen-sensitive patients at least, absorption must occur. It is possible that such absorption occurs to a greater extent in children. Some of the excellent results reported in children with this method of treatment would be difficult to explain on any other basis. Several cases of unequivocal attacks of asthma and hay fever following the ingestion of ragweed pollen were reported by Feinberg and his associates. These reactions also can be explained only on the basis of absorption of ragweed pollen from the gastrointestinal tract.

SEROLOGIC CHANGES FOLLOWING HYPODERMIC POLLEN TREATMENT

It is well established that allergic patients receiving parenteral treatment with pollen extracts develop measurable serologic changes. Levine and Coca[9] demonstrated by the method of serum dilution that an increase in reagin (skin-sensitizing antibody) of the patient's serum during treatment, often occurred. Schmidt and Lippard[10, 11] observed that, after patients received treatment, the serum acquired the ability to neutralize more antigen. Parallel titration of the skin-sensitizing antibody, using the serum dilution method, showed that in many of their patients the increased ability to neutralize antigen was accompanied by a rise in the titer of the skin-sensitizing antibody. They attributed the increased ability of the serum to neutralize ragweed antigen as due to the increase in skin-sensitizing antibody. However, in view of the experiments of Cooke, Barnard, Hebald, and Stull[12] and the subsequent work of Loveless,[13] it is evident that the increased ability of the post-treatment serum to neutralize ragweed antigen is due chiefly to the production of another antibody, the so-called inhibiting antibody or

blocking substance, and only partly, if at all, to the increase in the skin-sensitizing antibody.

Because of the inconclusive evidence of absorption of ingested pollen, it was felt that a study of the effect of oral pollen treatment, compared to hypodermic treatment, on the neutralizing capacity and the skin-sensitizing ability of the serum, might settle the question of enteral absorption. Children were selected for these experiments in view of the previously cited reports that better clinical results were obtained in children than in adults. It was hoped that, should serologic changes develop after oral pollen treatment, they would thus be more readily detected.

SELECTION OF PATIENTS FOR SEROLOGIC STUDY

Two groups of patients were studied. One received ragweed pollen extract hypodermically; the other was treated orally. The patients were children between the ages of 8 and 13 years who gave typical histories of hay fever during the ragweed season and were previously untreated. All gave good skin reactions to ragweed by the scratch method. No asthmatic patients were included in this study. In addition, we also excluded all cases complicated by other types of clinical pollen sensitivity or those patients who required desensitization for antigens other than ragweed. The children in both groups were, therefore, essentially of the same clinical type—patients with simple autumnal hay fever, previously untreated.

It was also necessary to exclude from this report, after preliminary tests, patients whose sera persistently showed no evidence of skin-sensitizing antibody since it was impossible to carry out the quantitative neutralization tests, by using the method to be described, in the absence of the skin-sensitizing antibody. These patients with an initial and persistent absence of skin-sensitizing antibody have, however, been studied serologically and will be the subject of an additional report. There were ten children in the orally treated group and thirteen in the hypodermically treated group who fulfilled the requirements as outlined and on whom sufficiently complete studies were made.

METHODS OF TREATMENT

1. *Oral Pollen Treatment.*—Our preliminary studies in the summer of 1938 indicated that a maximum dosage of 2 grains of ragweed pollen given twice a day (240,000 Noon pollen units per day) was productive of good results in eight of ten children treated. We therefore followed the same procedure. The initial dose was $\frac{1}{256}$ grain of mixed short and giant ragweed pollen. This dosage was doubled each day until 2 grains of the mixed ragweed pollens were given twice a day. This was continued until the onset of the pollen season at which time, in some cases, it was reduced to 1 or 2 grains once a day or every two or

three days, depending upon the individual patient. In most instances, the patients received the maximum dosage of ragweed pollen by mouth for several months prior to the pollen season, since it was our intention to produce the maximum serologic effect.

There were very few untoward reactions. These consisted of nausea or mild abdominal pain in a few of the children. In the majority of the patients, there was a complete absence of reactions to such large doses of ragweed pollen. This is in contradistinction to our experience and that of others in the treatment of adults by the oral method.

Most observers report a high incidence of gastrointestinal reactions in adults, ranging from 20 to 30 per cent. Occasional constitutional reactions characterized by asthma, hay fever, or urticaria have also been reported. In one of our adult patients a severe gastrointestinal reaction accompanied by gross hemorrhage from the bowel occurred. The surprising absence of all constitutional reactions and the exceedingly few and relatively mild gastrointestinal reactions in this group of children treated orally is worth remarking.

2. *Hypodermic Treatment.*—The hypodermic treatment was carried out with an extract of equal parts of short and giant ragweed prepared in 5 per cent alkaline glucose solution, using 0.4 per cent phenol as a preservative. The treatment extract was standardized on the weight-volume basis. An attempt was made to reach a dose in excess of 0.5 c.c. of 3 per cent pollen extract (30,000 Noon units per cubic centimeter). The dosages actually reached varied from 2,000 to 27,000 Noon pollen units. The top dosage was then continued until after the peak of the pollen season at three-to seven-day intervals, depending upon the individual patient.

EXPERIMENTAL METHODS

The technique described by Lippard and Schmidt[14] to titrate the capacity of serum to neutralize ragweed antigen was modified for use in these experiments. Their method was combined with the serum dilution method[15] for titrating the skin-sensitizing antibody (reagin). Both determinations were carried out simultaneously on each of the sera.

1. *Preparation of Serum.*—Sterile blood was obtained from patients having negative Kahn tests prior to and several times during the course of ragweed pollen treatment. The serum was separated under sterile precautions, stored in sterile rubber-capped vials in the icebox without the use of a preservative and used within one week.

2. *Preparation of Antigen-Serum Mixtures for the Titration of the Neutralizing Capacity of the Serum.*—Fresh extracts of defatted ragweed pollen were prepared monthly in 5 per cent alkalinized glucose fluid preserved with 0.4 per cent phenol. The extracts were standardized on the protein nitrogen content.[16] Stock dilutions of ragweed antigen were prepared as shown in the first column of Table II. One-

tenth cubic centimeter of each dilution was placed in sterile vials; to this was added 0.4 c.c. of the serum. The protein nitrogen content of ragweed antigen per cubic centimeter of the resultant mixtures is shown in the second column. The protein units* per cubic centimeters of the resultant mixtures are shown in the third column. Each cubic centimeter of the antigen-serum mixtures contained 0.8 c.c. of serum. The antigen-serum mixtures were allowed to stand in the icebox for twenty-four hours before injection.

TABLE II

NO.	STOCK DILUTIONS OF RAGWEED ANTIGEN	MG. RAGWEED PROTEIN NITROGEN PER C.C. IN THE ANTIGEN-SERUM MIXTURES	PROTEIN UNITS OF RAGWEED PER C.C. OF ANTIGEN-SERUM MIXTURES
1	0.000015	0.000003	0.3
2	0.00015	0.00003	3.0
3	0.0015	0.0003	30.0
4	0.003	0.0006	60.0
5	0.0075	0.0015	150.0
6	0.015	0.003	300.0
7	0.03	0.006	600.0
8	0.075	0.015	1500.0
9	0.15	0.03	3000.0

*Protein unit equals 0.00001 mg. of protein nitrogen.[16]

3. *Selection of Recipients.*—The recipients were children on the wards of the hospital between the ages of 8 and 12 years. These patients were convalescing from orthopedic procedures or had had pneumonia or other infections. All had been afebrile for several days and in most cases were ready for discharge from the hospital. Previous intracutaneous tests were done to eliminate recipients sensitive to ragweed. Two recipients were used simultaneously for each titration. They were so selected that their age and weight were approximately the same. No recipients were used for more than one titration.

4. *Injection of Antigen-Serum Mixtures.*—One-tenth cubic centimeter portions of the antigen-serum mixtures were injected at intervals of approximately 5 cm. into the skin of the right forearm of each of two recipients. It has been shown[17] that reactions to injections of antigen into the skin of the forearm are greater at sites near the elbow than at the wrist. The intracutaneous injections of the antigen-serum mixtures were therefore made so that the weaker concentrations of ragweed were given in the skin nearer the wrist, and the stronger concentrations of ragweed (the ones most likely to neutralize all the antibody contained in the serum) in ascending order toward the elbow. In this manner errors due to the tendency for reactions to occur more readily in the skin next to the elbow region were obviated.

5. *Serum Dilution Method for Titration of the Skin-Sensitizing Capacity of Serum.*—Dilutions of the serum were full strength, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128. One-tenth cubic centimeter of each dilu-

tion was injected into the skin of the left forearm of the recipients who received the antigen-serum mixtures in the right forearm. The dilutions were injected in order of strength, the weaker dilutions toward the elbow.

6. *Secondary Injection of Ragweed Antigen.*—A secondary injection of ragweed antigen was given twenty-four hours after the sites were prepared as indicated under 4 and 5. One and one-half cubic centimeters of a ragweed extract containing 0.5 per cent protein nitrogen per cubic centimeter were injected subcutaneously the day following the preparation of the above mentioned sites. In some larger children 2.0 c.c. of the ragweed extract were injected.

Our reason for not making the secondary injections of antigen directly into the locally sensitized sites, as is the technique of others, is that there is a frequent occurrence of questionable positive reactions by this method. These may result from the mechanical irritation of the sensitized site by the hypodermic needle or the injected fluid. Other irritative phenomena may occur, all of which tend to obscure the result and make the interpretation difficult. In our experience, if the secondary injection of antigen is made at a distant site, there is a clear and sharp distinction between the reacting and nonreacting locally sensitized sites.

The method of reading titrations was as follows:

1. *Neutralizing Capacity of the Serum.*—After the subcutaneous injection of ragweed antigen into the locally sensitized recipient, wheals appear within fifteen to seventy-five minutes at the sites of previous injections in which the mixture contained insufficient antigen to neutralize the skin-sensitizing antibody completely. In these areas the antibody remains free to sensitize the cells, and a reaction occurs when the antigen, which is absorbed into the circulation after subcutaneous injection, comes in contact with the sensitized tissues. Where complete neutralization of the antibody occurs, no reaction is observed. The dilution of the antigen, at which there is complete neutralization of the antibody without an excess of antigen, lies between the last reacting and the first nonreacting sites.

In recording the results of the neutralization tests we have omitted entirely the immediate reactions to the antigen-serum mixtures which occur at the time of intracutaneous injection and have noted only the reactions obtained after the secondary injection of ragweed antigen twenty-four hours later. The immediate reactions are no doubt of theoretical interest from the standpoint of the "blocking mechanism,"[12] but they are frequently difficult to evaluate or to differentiate from irritative phenomena.

2. *Skin-Sensitizing Antibody Titer.*—After subcutaneous injection of ragweed into the recipient, positive skin reactions appeared in from

fifteen to seventy-five minutes at the sites previously sensitized with progressive dilutions of serum. The end point is considered to be the greatest dilution of serum giving a positive reaction.

In recording the results of both the neutralization tests and the serum dilution tests no attempt was made to indicate the size of the reactions which appeared at the previously sensitized sites after the secondary injection of antigen. It seemed that no practical advantage was to be gained by this additional observation since the size of the positive reaction depended on many factors, including the quantitative variation in the ability of the skin to accept sensitization. It has been noted frequently that the skins of normal persons differ in the intensity of their reaction when sensitized with the same serum. For this reason, in the tables showing the results (Tables III, IV, and V), no quantitative differences in degree of positive reaction are shown for the reacting sites which are simply indicated by a plus sign.

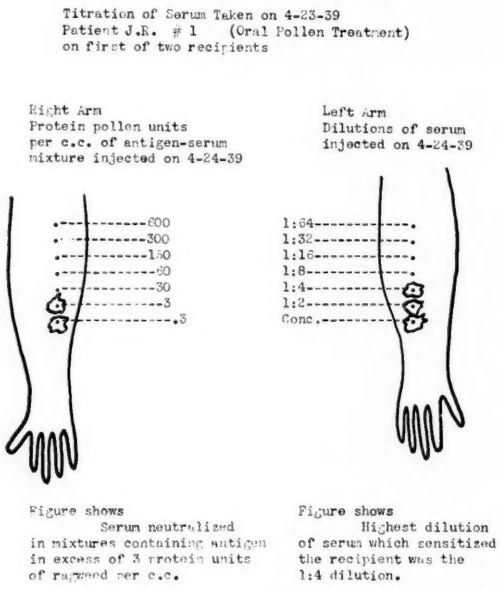

Fig. 1.

As previously noted, all titrations were done in duplicate on two recipients simultaneously. This eliminates the possibility of false results from the use of a person with nonreactive skin as a recipient. In the great majority of experiments, the end point of both the neutralizing capacity and skin-sensitizing ability of the sera were identical in both recipients. In the few cases that differed, the higher reading of the two was taken as the end point.

A schematic representation of the first titration of the serum of Case 1 (oral pollen group) on one of the two recipients used is shown in Fig. 1.

The results of all titrations on the serum taken at intervals from Case 1 of the group treated by the oral method is shown in Table III. It can be seen that the results are almost identical in both recipients. Whenever a difference occurred, the higher of the two readings was recorded as the end point. The dilution test on the serum taken on April 2, 1939, sensitized one recipient in 1:4 dilution, and the other in a 1:2 dilution. In the final tabulation (Table V) the 1:4 dilution was recorded as the last positive reacting site. The neutralization tests on the serum taken on July 25, 1939, gave the end point at 60 protein units in one recipient and 150 protein units in the other recipient. We considered the end point to be the higher of the two readings. In other words, it required a quantity of antigen slightly in excess of 150 protein units to neutralize 0.8 c.c. of the serum taken on that date. If the lower reading were recorded, any increase due to treatment would appear to be relatively greater.

TABLE III

TITRATIONS ON PATIENT J. R., NO. 1 ORAL

DATE	PROTEIN UNITS REQUIRED TO NEUTRALIZE 0.8 C.C. SERUM						TITER OF SKIN-SENSITIZING ANTIBODY BY SERUM DILUTION METHOD						
	0.3	3	30	60	150	300	1	½	¼	⅛	1/16	1/32	1/64
4/23/39													
1st recip.	+	+	0	0	0	0	+	+	+	0	0	0	0
2nd recip.	+	+	0	0	0	0	+	+	0	0	0	0	0
6/ 2/39													
1st recip.	+	+	+	+	0	0	+	+	+	0	0	0	0
2nd recip.	+	+	+	+	0	0	+	+	+	0	0	0	0
7/25/39													
1st recip.	+	+	+	+	0	0	+	+	+	+	+	+	0
2nd recip.	+	+	+	+	+	0	+	+	+	+	+	+	0
9/13/39													
1st recip.	+	+	+	+	0	0	+	+	+	+	+	+	0
2nd recip.	+	+	+	+	0	0	+	+	+	+	+	+	0

In five patients (Table IV, Patients 4, 8, and 9; Table V, Patients 2 and 8) the serum was titrated on two occasions from four to six weeks apart prior to initiating treatment. Longer control periods to determine the normal fluctuation of antibody might have been advisable. However, Schmidt, and Lippard[11] have shown that, in control periods up to five months prior to treatment, no significant change in antibody occurred. In the five patients referred to above, we also found insignificant pretreatment variations.

It has been shown[10] that, during a period of exposure to atmospheric pollen, the titer of the skin-sensitizing antibody and the neutralizing capacity of the serum increased moderately. We made titrations on most of these patients during and after the pollen seasons, but we have omitted such observations from this report since any changes in antibody content of the serum occurring after the onset of the ragweed season might be due to the summation of two effects, the effect of

TABLE IV

RESULTS OF NEUTRALIZATION TESTS AND SERUM DILUTION TESTS IN HYPODERMICALLY TREATED RAGWEED HAY FEVER PATIENTS

NO.	PATIENT	DATE SERUM TAKEN	DOSAGE REACHED (NOON POLLEN UNITS)	NEUTRALIZING CAPACITY OF THE SERUM. PROTEIN POLLEN UNITS PER C.C. ANTIGEN-SERUM MIXTURES							TITER SKIN-SENSITIZING ANTIBODY BY SERUM DILUTION METHOD								CLINICAL RESULTS
				0.3	3	30	60	150	300	600	1	½	¼	⅛	1/16	1/32	1/64	1/128	
1	M. G.	4/11/39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		7/17/39	9,000	+	+	0	0	0	0	0	+	+	0	0	0	0	0	0	75% relief
2	L. G.	4/ 2/39	0	+	+	+	0	0	0	0	+	+	+	+	+	0	0	0	
		8/ 1/39	7,000	+	+	+	+	0	0	0	+	+	+	+	+	+	0	0	Excellent
3	S. W.	4/ 7/39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		6/30/39	27,000	+	+	0	0	0	0	0	+	+	+	0	0	0	0	0	
		9/21/39	21,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Excellent
4	J. Q.	3/ 1/39	0	+	0	0	0	0	0	0	+	0	0	0	0	0	0	0	
		4/ 4/39	0	+	0	0	0	0	0	0	+	0	0	0	0	0	0	0	
		7/11/39	12,000	+	+	+	+	0	0	0	+	+	0	0	0	0	0	0	Excellent
5	G. I.	4/11/39	0	+	+	+	0	0	0	0	+	0	0	0	0	0	0	0	
		8/ 1/39	5,000	+	+	+	+	0	0	0	+	+	+	+	+	+	+	0	50% relief
6	G. C.	6/20/39	0	+	0	0	0	0	0	0	+	+	+	0	0	0	0	0	
		8/13/39	6,000	+	+	+	+	0	0	0	+	0	0	0	0	0	0	0	Excellent
7	T. P.	6/30/39	0	+	+	0	0	0	0	0	+	+	+	+	+	+	+	+	
		8/19/39	8,500	+	+	+	+	0	0	0	+	+	+	+	+	+	0	0	75% relief
8	D. R.	3/17/39	0	+	+	0	0	0	0	0	+	0	0	0	0	0	0	0	
		4/21/39	0	+	+	0	0	0	0	0	+	0	0	0	0	0	0	0	
		7/26/39	21,000	+	+	+	+	0	0	0	+	+	+	0	0	0	0	0	Excellent
9	J. R.	3/17/39	0	+	0	0	0	0	0	0	+	0	0	0	0	0	0	0	
		4/21/39	0	+	0	0	0	0	0	0	+	+	0	0	0	0	0	0	
		7/26/39	18,000	+	+	+	+	0	0	0	+	+	+	0	0	0	0	0	Excellent
10	N. V.	3/14/39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		6/23/39	2,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		8/10/39	18,000	+	+	+	+	+	0	0	+	+	+	+	+	+	0	0	50% relief
11	J. F.	3/21/39	0						0	0						0	0	0	
		7/25/39	21,000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50% relief
12	A. C.	3/17/39	0	+	0	0	0	0	0	0	+	+	0	0	0	0	0	0	
		7/26/39	18,000	+	+	0	0	0	0	0	+	0	0	0	0	0	0	0	Excellent
13	J. G.	4/11/39	0	+	+	+	0	0	0	0	+	+	+	0	0	0	0	0	
		8/ 1/39	7,000	+	+	0	0	0	0	0	+	+	+	+	+	0	0	0	75% relief

TABLE V

RESULTS OF NEUTRALIZATION AND SERUM DILUTION TESTS IN ORALLY TREATED RAGWEED HAY FEVER PATIENTS

NO.	PATIENT	DATE SERUM TAKEN	DOSAGE REACHED	NEUTRALIZING CAPACITY OF THE SERUM. PROTEIN POLLEN UNITS PER C.C. ANTIGEN-SERUM MIXTURES							TITER SKIN-SENSITIZING ANTIBODY BY SERUM DILUTION METHOD								REACTIONS AND CLINICAL RESULTS
				0.3	3	30	60	150	300	600	1	½	¼	⅛	1/16	1/32	1/64	1/128	
1	J. R.	4/23/39		+	+	0	0	0	0	0	+	+	+	0	0	0	0	0	No reactions
		6/ 2/39	2 Gr. b.i.d.	+	+	+	+	0	0	0	+	+	+	0	0	0	0	0	Excellent results
		7/25/39	2 Gr. b.i.d.	+	+	+	+	0	0	0	+	+	+	+	+	0	0	0	
2	B. T.	2/24/39		+	+	0	0	0	0	0	+	+	0	0	0	0	0	0	No reactions
		4/23/39		+	0	0	0	0	0	0	+	0	0	0	0	0	0	0	50% relief
		6/16/39	2 Gr. b.i.d.	+	+	+	+	0	0	0	+	+	+	0	0	0	0	0	
		7/21/39		+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	
3	J. P.	3/31/39		+	+	0	0	0	0	0	+	0	0	0	0	0	0	0	No reactions
		6/ 9/39	2 Gr. b.i.d.	+	+	0	0	0	0	0	+	+	+	0	0	0	0	0	Treatment discontinued
4	J. S.	5/12/39		+	+	0	0	0	0	0	+	+	+	0	0	0	0	0	Slight abdominal pain occasionally
		6/20/39	2 Gr. b.i.d.	+	+	0	0	0	0	0	+	+	+	+	+	+	0	0	
		7/25/39	2 Gr. b.i.d.	+	+	+	+	0	0	0	+	+	+	+	+	0	0	0	75% relief
5	D. A.	5/23/39		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Occasional nausea
		6/30/39	2 Gr. b.i.d.	+	+	0	0	0	0	0	+	+	+	+	+	+	0	0	No improvement
		7/21/39	2 Gr. b.i.d.	+	+	0	0	0	0	0	+	+	+	+	+	+	0	0	Oral treatment discontinued
		10/10/39	Discontinued 8/22	+	+	+	+	0	0	0	+	+	+	+	+	+	0	0	8/22 and injections begun
6		2/14/40		+	0	0	0	0	0	0	+	+	0	0	0	0	0	0	No reactions
		3/16/40	2 Gr. b.i.d.	+	+	0	0	0	0	0	+	+	+	+	+	0	0	0	75% relief
		5/12/40	2 Gr. b.i.d.	+	+	+	+	0	0	0	+	+	+	+	+	0	0	0	
7		4/23/40	2 Gr. b.i.d.	+	+	0	0	0	0	0	+	0	0	0	0	0	0	0	No reactions
		5/12/40	2 Gr. daily	+	+	0	0	0	0	0	+	+	+	+	+	0	0	0	
		6/ 2/40	1 Gr. daily	+	+	+	0	0	0	0	+	+	+	0	0	0	0	0	Excellent result
8	W. G.	1/23/40		+	+	+	+	0	0	0	+	+	+	+	+	0	0	0	Slight abdominal pain and occasional nausea if given more than 1 Gr. per day. 50% relief
		2/23/40		+	+	+	+	0	0	0	+	+	+	+	0	0	0	0	
		3/ 8/40		+	+	+	+	+	0	0	+	+	+	+	+	+	+	0	
		4/ 8/40		+	+	+	+	0	0	0	+	+	+	+	+	+	+	0	
		7/20/40		+	+	+	+	+	0	0	+	+	+	+	+	+	+	+	
9		3/16/39		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	No reactions
		5/12/39	1 Gr. b.i.d.	+	+	0	0	0	0	0	+	+	+	+	0	0	0	0	
		6/ 2/39	2 Gr. b.i.d.	+	+	+	+	+	0	0	+	+	+	+	+	0	0	0	75% relief
10		5/12/39		+	0	0	0	0	0	0	+	0	0	0	0	0	0	0	No reactions
		6/20/39	2 Gr. b.i.d.	+	0	0	0	0	0	0	+	+	+	0	0	0	0	0	
		7/25/39	2 Gr. b.i.d.	+	+	+	0	0	0	0	+	+	+	0	0	0	0	0	75% relief

treatment and the effect of the inhalation of atmospheric pollen. The subsequent titrations of the sera of these patients will be reported in a future publication.

RESULTS

The results of the titrations in the thirteen hypodermically treated patients are shown in Table IV. Eleven patients showed an increase in the neutralizing capacity of the serum. Nine showed an increase in the skin-sensitizing ability of the serum. These results confirm previous reports that hypodermic treatment produces an increase in the neutralizing capacity of the serum and an increase in the skin-sensitizing antibody. Sherman and his associates[18] have recently shown that during the first several months of treatment there is a tendency for the skin-sensitizing antibody to increase, but after prolonged treatment a decrease usually occurs.

The results of these tests on the orally treated group are shown in Table V. It is obvious that oral pollen treatment in children produces similar antibody changes to that occurring in the hypodermically treated group. Nine patients showed an increase in the neutralizing capacity of the serum, and all ten showed an increase in the skin-sensitizing ability of the serum. In the majority of the patients treated in both groups, there was a simultaneous increase in the neutralizing capacity of the serum and the skin-sensitizing antibody. However, such increases were not always proportionate, and in a few cases an increase in the skin-sensitizing antibody titer occurred with no increase in the neutralizing capacity of the serum or was accompanied by a decrease. In a few cases the neutralizing capacity of the serum increased in conjunction with a decrease of its skin-sensitizing ability. These findings confirm by indirect evidence the conclusion of others that the skin-sensitizing ability of the serum and its neutralizing capacity are the functions of two distinct antibodies.

These series of cases are too small to make comparisons between the clinical value of the oral and hypodermic methods of treatment. There is some indication from these results that the extent of relief and the percentage of patients completely relieved were somewhat less in the orally treated group than in the hypodermically treated group. It is noteworthy that three of the ten orally treated children had mild gastrointestinal symptoms. There were no severe reactions such as those seen in adults.

The primary purpose of this investigation was to determine whether ingested pollen is absorbed in sufficient quantities to effect serologic changes approximating those occurring in hypodermically treated patients. We believe that our results warrant an affirmative answer. It is interesting to speculate as to the reason ingested pollen in doses up to 5 Gm. failed to produce positive reactions at previously sensitized

skin sites. The possibility is suggested that the rate of absorption from the gastrointestinal tract is much too slow to produce sufficient antigen concentration in the blood to react locally at the sensitized sites.

SUMMARY

1. The ingestion of 5 Gm. of ragweed pollen in five children did not produce positive reactions at skin sites previously injected with ragweed skin-sensitizing antibody.

2. In thirteen patients treated hypodermically with doses from 5,000 to 27,000 Noon pollen units, an increase in the neutralizing and skin-sensitizing capacity of the serum occurred to a measurable degree in most cases.

3. In the patients treated with ragweed pollen orally with doses up to 2 grains a day (240,000 Noon pollen units per day), similar increases in the neutralizing capacity and the skin-sensitizing antibody of the serum occurred.

4. Evidence is presented by serologic changes that in children ragweed pollen is absorbed from the gastrointestinal tract.

REFERENCES

1. Feinberg, S. M., Foran, F. L., Lichtenstein, M. R., Padnos, E., Rappaport, B. Z., Sheldon, J., and Zeller, M.: Oral Pollen Therapy in Ragweed Pollinosis, J. A. M. A. **115**: 23, 1940.
2. Bohner, C. B.: Discussion of paper by Zeller.
3. Thommen, A. A.: Asthma and Hay Fever in Theory and Practice, Springfield, Ill., 1931, Charles C Thomas, p. 764.
4. Black, J. H.: The Oral Administration of Pollen: A Clinical Report, J. Lab. & Clin. Med. **13**: 709, 1928.
5. Bernstein, C., Jr., and Kirsner, J. B.: Oral Pollen Therapy, J. ALLERGY **8**: 221, 1937.
6. Bernstein, T. B., and Feinberg, S. M.: Oral Ragweed Pollen Therapy: Clinical Results and Experiments on Gastrointestinal Absorption, Arch. Int. Med. **62**: 297, 1938.
7. Zeller, M.: Oral Ragweed Pollen Therapy, J. ALLERGY **10**: 579, 1939.
8. London, M.: Combined Oral and Subcutaneous Treatment for Ragweed Pollinosis, J. ALLERGY **10**: 453, 1939.
9. Levine, P., and Coca, A. F.: Specific Treatment of Hay Fever: Nature of the Alleviating Effect, J. Immunol. **11**: 449, 1926.
10. Schmidt, W. M., and Lippard, V. W.: Human Passive Transfer Antibody. II. Neutralization of Antigen, Am. J. Dis. Child. **56**: 550, 1938.
11. Schmidt, W. M., and Lippard, V. W.: Human Passive Transfer Antibody. III. Serial Titrations on Treated and Untreated Patients With Hay Fever, Am. J. Dis. Child. **56**: 550, 1938.
12. Cooke, R. A., Barnard, J. H., Hebald, S., and Stull, A.: Serological Evidence of Immunity With Coexisting Sensitization in a Type of Human Allergy (Hay Fever), J. Exper. Med. **62**: 733, 1935.
13. Loveless, M. H.: Immunological Studies of Pollinosis. I. The Presence of Two Antibodies Related to the Same Pollen-Antigen in the Serum of Treated Hay Fever Patients, J. Immunol. **38**: 25, 1940.
14. Lippard, V. W., and Schmidt, W. M.: Human Passive Transfer Antibody. I. Titration by Neutralization, Am. J. Dis. Child. **521**: 288, 1937.
15. Coca, A. F., and Grove, E. F.: Studies in Hypersensitiveness, XIII, A Study of the Atopic Reagin, J. Immunol. **10**: 445, 1925.
16. Cooke, R. A., and Stull, A.: The Preparation and Standardization of Pollen Extracts for the Treatment of Hay Fever, J. ALLERGY **4**: 87, 1933.

17. Bosman, K.: Pertinent Factors Influencing Comparative Skin Tests on the Arm, J. ALLERGY, 7: 39, 1935.
18. Sherman, W. B., Stull, A., and Cooke, R. A.: Serologic Changes in Hay Fever Cases Treated Over a Period of Years, J. ALLERGY 2: 225, 1940.

DISCUSSION

DR. FRANCIS M. RACKEMANN, Boston.—I should like to make a comment on the serologic reactions that have been reported.

At a meeting of the Society for the Study of Asthma and Allied Conditions, Miss Scully and I reported our efforts to find the mechanism of the blocking antibody in patients with hay fever. We took the serum of the patients and used mixtures of this serum with ragweed to sensitize sites on normal recipients. The next day the sites were tested with ragweed, and the concentration of ragweed necessary to abolish the reaction was noted. We found that, in the case of serum drawn after the treatment, a larger amount of ragweed was necessary to block the reaction in the treated sites than in the case where the serum was drawn before treatment.

As Dr. Cooke and later Dr. Loveless have shown the difference is to be explained by the presence of something which keeps the ragweed and the serum antibody apart. As Dr. Loveless has put it, "the blocking antibody competes with the sensitizing antibody for the ragweed."

Now, there was, as I say, a difference in the concentration of ragweed necessary for the two types of serum, taken before and after treatment; there was evidence of the presence of the blocking antibody in the post-treatment serum.

The interesting thing, however, was that the concentration, the amount of this post-treatment antibody, was not proportional to the success of that treatment.

In the discussion of our paper, Dr. Cooke helped us a good deal by referring us back to the chemical studies of Stull, and you remember that Stull showed that Fraction 1 of the ragweed was an albumin precipitated by half-saturated ammonium sulphate, whereas Fraction 2 was a globulin which did not come down until the solution was fully saturated. The presence of these two rather distinct chemical substances complicates the picture because it is quite likely that different sorts of antibodies are developed to each of these two chemical fractions.

At the present time, when whole ragweed is used, as Dr. Levin mentioned, any results are always very difficult to interpret, and we cannot explain them finally until a much purer chemical solution of the ragweed is at hand.

DR. MARION B. SULZBERGER, New York City.—If my understanding is correct, the experiments of Drs. Levin and Shulsky have demonstrated once again that the gastrointestinal absorption of so-called protein allergens is more rapid in children, in young individuals, than in adults. That the rate of absorption of substances of this kind was more rapid in infants was first brought out by Schloss, Lippard, Anderson, and a great many other workers, many years ago.

The second point which the present interesting studies again bring to light is that, when one administers certain protein allergens, one produces an increase in skin-sensitizing antibodies (Prausnitz-Kuestner antibodies) and an increase in neutralizing antibodies, that is, in antibodies not capable of sensitizing the skin but capable of neutralizing the specific antigen in vitro.

As you all know, Coca, and, I believe, also Cooke and his school many years ago proved that in successfully treated hay fever cases there was an increase of Prausnitz-Kuestner or skin-sensitizing reagin rather than a decrease; often there was practically no change or, if there was any change, it was not a decreased reagin content of the serum, but rather an increased content after the successful specific treatment.

Yet other antibodies which neutralize the antigen in question in vitro are increased after successful desensitization treatment with specific antigens. This fact and these antibodies first demonstrated, I believe, by Storm van Leywen and co-workers, and shortly thereafter by W. Jadassohn, and later by Robert Cooke and his school, and Dr. Lovelace, and Dr. Milton Cohen, demonstrated the presence of increased amounts of neutralizing antibodies after specific treatment.

I think it is preferable to continue to speak of these antibodies simply as "neutralizing antibodies." That brings this phenomenon into line with other well-known phenomena of reaction between an antigen and its specific antibody, instead of bringing it into the vague and mysterious realm of entirely unrelated and unknown phenomena as the physical blocking first demonstrated by Cooke. This phenomenon noted by Cooke is neutralization of antigen by a specific antibody as I first pointed out in a discussion of Dr. Cooke's paper at one of these meetings several years ago.

Another point of interest in Dr. Levin's work is the demonstration of variability in the gastrointestinal rate of absorption of protein allergens. Dr. R. L. Baer, Dr. Rudolph Hecht, and I carried out some as yet unreported studies on the rate of absorption of pollen given by mouth. These were simple experiments in which we simply put a passive transfer serum in the skin of our own arms and then swallowed quantities of the pollen. In this way we attempted to find out whether it was being absorbed and how soon the passive transfer site reacted, and thus to discover whether and at approximately what rate significant amounts of pollen were being absorbed from our gastrointestinal tracts. In my own arm I got a beautiful, severely pruritic, and enormous urticarial reaction after fifteen to twenty-five minutes, repeatedly, with various pollens. The sensitized sites showed the classic form of response due to the gastrointestinal absorption of the pollen.

But in the arms of Baer and Hecht we never could produce a reaction of a passively sensitized site. In other words, in them, either the gastrointestinal absorption was not sufficiently rapid or the pollen was changed in some way before it got to that site, so that no reaction occurred; whereas in me the absorption was quick, and enough of the unchanged pollen antigen got to the skin site to produce the reaction. Having established this variation conclusively, we next tried to study the phenomenon and to find out why there was a difference between us.

For example, one morning bright and early we all lined up to have gastric analyses done, and it was a very amusing scene I am sure. We thought perhaps the hydrochloric acid content of the stomach might have something to do with the difference in rate of absorption; and while it was true that my own hydrochloric acid content was very low and that I did not have any on the second test without histamine, still we did not think the difference in acidity was sufficient to account for the tremendous difference in rate of absorption. Moreover, we then established that the administration of acid or alkali with the pollen did

not materially influence the absorption of pollen in our three cases. In the end, after many and varied experiments we had to postpone any further attempts to account for the great variation in the rate of gastro-intestinal absorption of pollen in the case of myself on the one hand and Baer and Hecht on the other. We had to content ourselves by saying that, although I was their senior by many years, nevertheless I seemed to have an absorption rate of the infantile type of stomach, whereas they had rates of the adult type.

DR. HORACE S. BALDWIN, New York City.—In the discussion of this antibody Dr. Levin has talked about, I think it is worth while reiterating what Dr. Sulzberger has said. It is not a blocking substance for which there is no well-recognized immunologic parallel, but a definite neutralizing antibody which conforms to definite immunologic principles. It corresponds to other artificially produced antibodies, and it would seem to me proper for us as immunologists to recognize that fact and clarify our thought on this matter. If we recognize this as a definite neutralizing antibody, we should use that terminology in referring to it.

Dr. Loveless is here, and I think she could add something to this discussion.

DR. MARY HEWITT LOVELESS, New York, N. Y.—I agree with Dr. Baldwin and Dr. Sulzberger in their objection to the terms "blocking" and "inhibiting." They are descriptive in that they immediately bring to mind the picture of an absent wheal and thus remind us that this antibody combines with its antigen without giving rise to tissue irritation, whereas urticaria always results when *reagin* combines with the same antigen. Nevertheless, the terms imply that the induced antibody exerts its influence upon the reagin-antigen complex, whereas we now know that the entire phenomenon is due to the ready inactivation of *antigen* by the induced antibody. This inactivation occurs even though reagin is likewise present and competing against the induced antibody for the common antigen.

I do not think that the term "neutralizing" is particularly much better as a means of differentiating the induced from the reaginic antibody, because no one has proved that the reagin does not also neutralize its antigen. My feeling is that it does neutralize the antigen, just as does the other antibody. In the ultimate analysis, the best description I could devise for the so-called "blocking" antibody was to refer to it as the "thermostable" antibody, because in this way it can be distinguished from the thermolabile reagin.

The origin of the idea that reagin is a nonneutralizing antibody goes back to some observations by Dr. Coca. He mixed reaginic serum and antigen in vitro and then tested the mixture in normal skin and in sensitive skin. Urticarial wheals occurred in each instance. Dr. Coca concluded that, if whealing occurred in sensitive skin, there must be unneutralized antigen available. In the case of the reaction in normal skin, however, he assumed that it was attributable to a combination of the antigen with the reagin he had added to the test tube. It seems to me simpler to make this same assumption for the response in the sensitive skin also. In this event, one has yet to prove that the reagin in the test tube failed to bind the antigen. Until we can devise a better type of experiment to test the theory, it would seem logical to assume that both

antibodies neutralize the antigen in combining with it. Until we know more about the induced antibody, it would seem best to refer to it simply as the "thermostable" antibody.

DR. LEVIN.—In presenting the paper as fast as I did, several interesting findings in connection with this work were necessarily left out because of lack of time. One of these is the confirmation of work done by others that the inhalation of atmospheric pollen produces definite increase in the antibodies. We made many such observations but have omitted them from this presentation since it was felt that it would serve only to confuse the issue as to whether pollen was absorbed from the gastrointestinal tract or not. Titrations made after the appearance of pollen in the atmosphere will be presented in a subsequent paper.

In regard to the point raised by Dr. Sulzberger, we have no evidence that absorption of pollen from the gastrointestinal tract occurs in adults. We have shown, I believe, that such absorption does occur in children. The work should be repeated on adults to see whether the results are similar. There is a possibility that differences will be found in the absorption rate of such material from the gastrointestinal tract in children and adults.

A CLINICAL STUDY OF HISTAMINE AZOPROTEIN IN ALLERGIC DISEASE

A Preliminary Report

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THE histamine theory of anaphylaxis and the application of this hypothesis as an explanation of allergic reactions are so well known at the present time that an extensive review of the subject as an introduction to this report seems unnecessary. Ever since Dale and Laidlaw[1] observed in 1910 that the symptoms of anaphylactic shock were similar to those of histamine poisoning, a great many investigations have been conducted in an effort to establish the validity of the concept that anaphylactic and allergic reactions were mainly, if not entirely, caused by histamine released from tissue cells by an antigen-antibody reaction. The problem has been reviewed recently in an excellent manner by Farmer.[2] Although no definite proof of the theory has been established, and some workers believe the importance of histamine has been exaggerated, there seems to be little question but that it plays some role in the anaphylactic reaction and probably in the idiosyncrasies.

Attempts to induce desensitization or refractoriness to histamine by injection of increasing doses of the compound were tried by Ramirez and St. George.[3] Since their report, a number of other workers have published articles on this method of therapy in urticaria, physical allergy, bronchial asthma, hay fever, and migraine. The number of cases studied has been small, and the results are not clear cut. Similar conflicting conclusions have been reported in anaphylaxis following animal experimentation, although the data given by Farmer[4] seem to justify his conclusion that continued administration of histamine to guinea pigs leads to some degree of refractoriness.

This type of approach does not seem to be too promising. Histamine is detoxified quickly in the animal body, and there appears to be no good reason for hoping it could induce a refractory condition unless, as has been suggested, it might stimulate the production of histaminase. Since histamine will combine with protein in vitro, it might join with

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tissue proteins to form an antigenic complex, but, so far as we know, no one has suggested this or attempted to prove it.

The idea of using histaminase to neutralize excess histamine would appear to be rational, except for the fact that the action of this enzyme in vitro is very slow. Attempts to prove its efficacy in abolishing anaphylaxis in animals have been inconclusive; the positive results reported by Karady and Browne[5] have not been confirmed.[6] Extensive clinical tests with extracts of kidney and intestinal mucosa containing histaminase have been conducted, but the evidence reported is hardly conclusive; the use of such extracts is open to the objection that they contain other compounds (than histaminase) which may contribute to any beneficial results obtained.

It seemed to us that a rational approach to the problem might be one based on the fundamental discoveries of Landsteiner[7] regarding immunologic specificity. If histamine could be combined with a protein, it might form an antigenic complex which would engender antibodies whose specificity would be determined by the histamine (hapten) portion of the molecule. Such antibodies might be able to neutralize any excess histamine released after an antigen-antibody reaction and thus prevent the development of the symptoms of anaphylactic or allergic reactions even though the antigen-antibody reaction had occurred. In other words, one might be able to build up an active immunity with histamine, and thus a nonspecific immunity to allergic reactions which would not be based on immunization or desensitization with one or more specific allergens.

Since we started this investigation, several reports of experimental work based on the same general principles have been published. Clutton and his associates[8] prepared thyroxin globulins and obtained antisera which inhibited the metabolic effects of thyroxin subsequently injected into rats. Butler and his associates[9] synthesized aspirin globulins. Antisera to these complexes seemed to prevent the alleviation of pyrexia that aspirin normally would produce in animals. Strychnine-protein antigens have been described by Hooker and Boyd,[10] and adrenalin azoprotein, by Went and Kesztyus.[11] Strauss and Spain[12] have conjugated ragweed pollen extracts. In most cases the serologic evidence indicated clearly that these pharmacologically active compounds were functioning in the artificial antigens as determinants of immunologic specificity, but, so far as we know, clinical tests of such products have not been reported.

EXPERIMENTAL DATA

1. Chemistry.—We first tried the synthesis used so frequently by Landsteiner to obtain artificial antigens, namely, the coupling of a simple chemical compound to a protein through an azo linkage. This

was accomplished by condensing histamine with p-nitrobenzoylchloride, reducing the nitro group with ferrous sulfate and ammonia, then diazotizing and coupling with protein (Fig. 1).

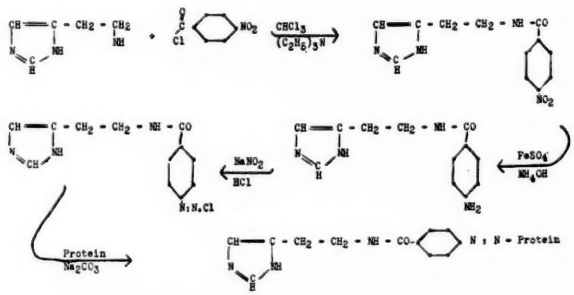

Fig. 1.—Synthesis of histamine azoprotein.

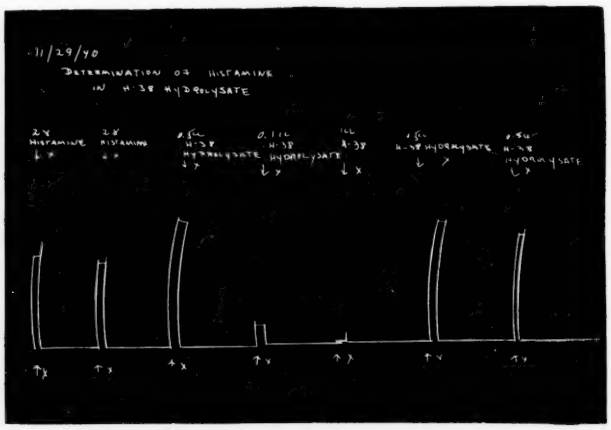

Fig. 2.—Determination of histamine in histamine-azo-despeciated horse serum globulin (H-38) before and after hydrolysis.

The choice of protein was based on the realization that, if our hypothesis proved faulty, we would not wish to risk sensitization or shock of allergic patients by using native proteins, such as horse serum, even though these probably would be denatured during the coupling process. Accordingly, we initially employed casein, which is supposed to be only infrequently responsible for allergic reactions in man. Histamine azocasein gave fairly satisfactory results in animals, but preliminary clinical trials were not at all encouraging. We then changed to horse serum globulin from which the horse serum specificity had been largely eliminated by the "despeciation" process described by Coghill and his associates.[13] With antigens made from these altered proteins, no matter how long the interval between injections, we have not as yet seen any anaphylactic or allergic reaction in animals or man. How much of this is due to denaturation during the synthesis, or to the

weak antigenicity of the despeciated globulins or perhaps to an actual protective mechanism stimulated by the histamine antigen, is still uncertain.

The complex protein contains practically no free histamine, as shown by testing it on the guinea pig ileum in the Schultz-Dale apparatus. After hydrolysis with concentrated hydrochloric acid, however, the histamine is liberated and can be assayed roughly by this method (Fig. 2). The total amount of histamine bound to the protein can be determined accurately either by absorbing it from hydrolysates on permutit, or by isolating it quantitatively as the picrate. By these methods we have found that some of our 2 per cent antigen solutions contain as much as 1 mg. of histamine (as the free base) per cubic centimeter.

The details of the proof of the immunologic specificity of histamine-azo-despeciated horse serum globulin and the results of experiments with laboratory animals will be reported in a separate communication.

We have coupled histamine to proteins by a variety of methods other than through the azo linkage. These include (a) isocyanohistamine (method used by Hopkins and Wormall), (b) histamine-benzidine protein, and (c) chloracetylhistamine protein. Poor yields, however, were obtained in most of these syntheses, and, since only a limited amount of immunologic work has been conducted with such compounds, details will be reported later in a separate communication.

A group of seventy-six patients has been selected for this study. In order to exclude intercurrent or other complicating diseases, all patients received a complete physical examination, a routine urinalysis, and a blood Kahn test for syphilis. Stereoscopic posterior-anterior roentgenograms of the chest were obtained on all asthmatic patients. Complete blood counts were performed in the majority of instances. We were able to produce symptoms in those patients selected for study by exposing them to specific excitants. From the group, thirty-nine representative cases of atopic eczema, contact dermatitis, physical allergy, seasonal hay fever, perennial allergic rhinitis, gastrointestinal allergy, and migrainous headaches are listed in Table I. Many of the subjects manifested two or more types of allergic symptoms. The age, sex, occupation, and results of treatment are given for each subject (Table I). Histamine azoprotein has been the only form of treatment, except in the atopic eczema and contact dermatitis groups where proper local therapy was permitted.

The histamine azoprotein was routinely tested intracutaneously in doses of 0.02 c.c. Some of the early (thirty-minute) reactions consisted of a wheal from 2 mm. to 4 mm. in diameter with a surrounding flare of 1 mm. to 2 mm. in width. In nine instances there was a delayed tuberculin type of reaction which measured from 8 mm. to 25 mm. in diameter. In the absence of a delayed reaction the first treatment has consisted

TABLE I

NO.	PATIENT	AGE (YEARS)	SEX	OCCUPATION	DIAGNOSIS	CLINICAL SENSITIVITY	DURATION OF TREATMENT (MONTHS)	HIGHEST DOSE OF HISTAMINE AZOPROTEIN (C.C. OF 1:10)	RESULTS
1	W. H.	26	F	Nurse	Contact dermatitis	Mercurials	3.5	0.2	Good
2	H. B.	29	F	Teacher		Dyes	11.0	0.8	Excellent
3	E. B.	21	M	Butcher		Lacquer	5.0	0.4	No relief
4	M. C.	32	F	Housewife	Contact dermatitis, gastrointestinal allergy, and allergic rhinitis	Nickel	19.0	1.0	Good
5	C. R.	18	M	Student	Contact dermatitis	Dyes	9.0	0.8	
6	E. S.	30	M	Clerk		Soaps	4.0	0.3	No benefit
7	J. K.	14	F	Student		?	3.0	0.3	?
8	V. A.	33	F	Housewife		Multiple	1.0	0.1	No benefit
9	J. W.	26	F	Housewife		Dyes	9.0	0.8	Excellent
10	E. B.	42	M	Furrier			3.0	0.2	
11	R. S.	67	M	Pharmacist		? Mercurials	2.5	0.4	No benefit
12	N. D.	28	M	Mechanic		Oils	4.5	1.0	Excellent
13	J. A.	43	F		Atopic eczema	Dust, karaya gum, and foods	1.0	0.1	No benefit
14	L. B.	30	F	Nurse		Multiple	1.0	0.2	Poor
15	G. S.	17	M	Student		Housedust, multiple	7.0	0.5	Excellent
16	C. E.	29	M	Physician		Multiple	6.5	0.3	Fair
17	G. W.	19	M	Schoolboy			4.5	0.1	

18	C. D.	27	F	Housewife			3.0	0.3	Excellent
19	M. K.	15	F	Student		Housedust, multiple	2.5	0.2	
20	F. J.	18	M	Student		Multiple	5.0	0.4	Fair
21	E. C.	48	F	Housewife	Asthma	Cold	2.5	0.1	Complete relief
22	C. M.	55	F			Cold, orange, grapefruit	6.0	0.5	
23	K. D.	19	F	Clerk	Urticaria	Heat and exercise	4.0	0.5	
24	L. R.	37	F	Housewife		Sunlight	20.0	1.2	Excellent
25	H. B.	46	M	Factory	Asthma	Multiple	13.0	0.2 (I.D.)	Complete relief
26	A. H.	53	M	Teacher	Asthma and perennial allergic rhinitis		2.0	0.3	No benefit
27	M. H.	23	F	Housework			6.0	0.7	
28	E. J.	35	F	Social worker	Seasonal asthma and hay fever	Pollens, dusts, and fungi	21.0	1.0	No asthma; good relief from hay fever
29	I. K.	52	M	Unemployed	Asthma	Food and dust	4.0	0.8	Temporary
30	V. M.	29	F	Housewife	Asthma and hay fever	Pollens	22.0	1.0	Excellent
31	H. W.	22	F	Unemployed	Asthma and perennial hay fever	Dust infection	5.0	1.0	No relief
32	D. F.	27	F	Housewife	Asthma	Multiple	3.5	0.3	No benefit
33	B. B.	17	F	Schoolgirl	Allergic rhinitis	Housedust	1.5	0.2	Partial relief while being treated
34	M. B.	43	F	Clerical		Multiple	7.0	1.0	No benefit
35	E. H.	39	F	Housewife			3.0	0.6	Fair
36	M. N.	31	F			Housedust	12.0	0.8	Complete
37	S. L.	29	M	Chemist	Urticaria	Acute infections	3.5	0.5	No relief
38	D. S.	31	F	Housewife	Gastrointestinal allergy	Wheat and potato	4.0	0.6	Excellent
39	G. W.	43	M	Mechanic	Migrainous headache	?	3.0	1.0	No benefit

I.D., Intradermally.

of 0.05 c.c. of undiluted histamine azoprotein subcutaneously, in the deltoid area. Subsequent doses were given twice weekly with an increase of 0.05 c.c. each time until symptomatic relief was obtained, or until a 1.0 c.c. dosage was reached. In those subjects who had a delayed type of reaction the initial dosage was greatly reduced, and subsequent increases were guided by symptomatic improvement or local reaction.

CASE REPORTS

The following case histories are illustrative of the successfully treated patients.

CASE 24.—L. R., a white female, housewife, aged 37 years, was first seen in the Medical Department of the University Hospital on Sept. 13, 1939, because of an urticarial eruption. She had been in good health until July, 1931, when she first experienced characteristic hay fever which disappeared in the late summer. Since 1931 there has been a yearly seasonal recurrence of her hay fever. In the summer of 1934 she first noticed white blotches on the exposed surfaces. The eruption was not particularly troublesome until the late summer of 1934, at which time she was exposed to considerable sunlight, developed a generalized urticarial eruption, and went into a state of unconsciousness. Since the latter episode she has noticed prompt appearance of urticaria on exposure to sunlight, and it had become so troublesome that she confined her activities to the inside of her home from sunrise to dusk. The past history and family history were not contributory.

The physical examination revealed a slightly overweight, adult, white female. There were white, elevated, pea- to dime-sized serpiginous lesions over the face, forehead, neck, wrists, and legs below the knee to the shoetops. The remainder of the examination was negative.

Laboratory Data.—The Kahn test for syphilis and urinalysis were negative. A complete blood count was negative. Urticarial lesions could be produced anywhere on the body surface with 25 per cent of an erythema dose from a mercury quartz lamp (3,000 to 3,500 Angstrom units) or carbon arc light. Serum was transferred to a nonlight-sensitive person's skin. Exposure of the serum-injected area of skin with 25 per cent of an erythema dose from a mercury quartz lamp caused mild erythema and itching. We were unable to reproduce the urticaria with 100 per cent of an erythema dose from the mercury quartz lamp after the patient had been treated for eleven months. Cutaneous tests were positive to June grass, timothy, orchard grass, and ragweed.

CASE 10.—E. B., a white, Jewish male, aged 42 years, a fur buyer by occupation, was first seen in the Dermatology Department of the University Hospital on Nov. 2, 1940, because of a skin eruption. He had developed an eruption on his hands five years previously. Since the onset he has always had some degree of skin involvement, most marked on the hands, but at times involving the face, eyelids, neck, and lower portion of the forearms. Some improvement occurred during vacations, but he was never away from work longer than three or four days.

The physical examination revealed an averaged-sized white male. The skin of the entire surface of the hands, wrists, eyelids, face, and neck showed a red, edematous, weeping, diffuse involvement. The remainder of the examination was of no diagnostic importance.

Laboratory Data.—The blood Kahn test and urinalysis were negative. The white blood cell count on admission was 12,500 with a polymorphonuclear leucocyte

differential of 76 per cent. Patch tests with a 2 per cent paraphenylendiamine in white vaseline gave a marked positive vesiculated lesion after eighteen hours. Several darkly dyed furs gave positive patch test reactions.

Clinical Course.—After four days of hospitalization with the usual topical treatment and histamine azoprotein the lesions were greatly improved. He then was permitted to return to work but continued with his local treatment and histamine azoprotein. After six weeks the lesions were completely healed in spite of the fact that he continued to handle the same dyes.

We have not observed any important local or systemic reactions following the use of histamine azoprotein. In one case there was mild flushing of the face, an increase in cardiac rate, and slight fall in blood pressure immediately following the administration of the compound. This reaction was very transitory, and subsequent administrations of larger doses of the material were uneventful. In the majority of the patients the area at the site of injection has shown a mild inflammatory reaction which disappeared within three to seven days. In four cases hard, nontender, pea-sized nodules at the site of injection have required two to three months for disappearance. The material has been given to horse serum-sensitive patients without reactions. In several patients treatment has been interrupted for intervals of thirty to ninety days. Subsequent larger doses of the compound did not produce allergic disturbances.

In order to test the possibility that administration of histamine azoprotein produced a histamine-neutralizing agent, the following two experiments were made.

Blood sera were collected from seven patients who had received treatment with histamine azoprotein. The serum was then incubated under sterile technique with 0.1 mg. of histamine phosphate for twenty-four hours at 37° C. Each histamine-plus-serum sample was then injected into three nonallergic people. In all but one instance there was a typical histamine wheal. Incubated serum controls from the same subjects failed to give the whealing reaction.

Five blood sera from patients treated with histamine azoprotein were injected into nonallergic patients. Three days later histamine phosphate (0.02 mg.) was injected into the blood sera transfer sites. Typical wheals with flare developed immediately.

We carried out two experiments to see if a gross amount of histamine was liberated from the conjugated histamine azoprotein molecule. The first consisted of repeated gastric analyses on four subjects. There was no rise in gastric acidity after the subcutaneous administration of 1.0 c.c. of histamine azoprotein, although these same patients showed a marked rise in gastric acidity with 0.3 mg. of histamine phosphate. Further evidence against appreciable liberation of histamine was the fact that neither the systolic nor the diastolic blood pressure changed after the administration of the histamine azoprotein.

Two patients (Cases 2 and 10) have shown a marked progressive quantitative reduction in their patch test reaction to paraphenylendiamine hydrobromide (the dye responsible for their contact eruptions) subsequent to treatment with the histamine azoprotein.

Six seasonal hay fever patients have been treated with histamine azocasein. None of these patients showed subjective or objective evidence of improvement. We believe that this is some evidence in favor of a specific effect of histamine-azo-despeciated horse serum globulin.

DISCUSSION

We are unable to find any correlation between the dosage of histamine azoprotein and the therapeutic results. The age and sex of the patient and duration of symptoms do not materially influence the prognosis. It does appear, however, that the type of allergic manifestation influences the efficacy of the histamine antigen. The best results were obtained in the physical allergy and skin hypersensitive groups. Two asthmatic patients (Cases 21 and 22), in whom asthma could be induced by exposure to cold, were relieved by three weeks of treatment. Asthma can no longer be produced in these two subjects. One patient (Case 24), who was sufficiently sensitive to sunlight to require confinement to her home during the daytime, is now able to do as she desires.

Although our group of persons with contact dermatitis is very small and the etiological causes limited to but a few substances, it would appear that histamine azoprotein in conjunction with local treatment has more to offer than any other form of approach known to us.

The atopic eczema patients as a group seemed to show definite benefit from histamine azoprotein therapy.

The seasonal ragweed hay fever patients treated with the histamine-azo-despeciated horse serum globulin as a group were benefited as much as were similar control patients receiving specific ragweed desensitization in 1940. It must be recognized, however, that the ragweed season was exceptionally light and that our series of cases was too small to evaluate adequately. We recognize that histamine-azo-despeciated horse serum globulin is no panacea in the treatment of all allergic diseases. We do believe, however, that the results so far are sufficiently encouraging to justify continuation of the study.

REFERENCES

1. Dale, H. H., and Laidlaw, P. P.: J. Physiol. **41**: 318, 1910.
2. Farmer, L.: Bull. New York Academy Med. **16**: 618, 1940.
3. Ramirez, M., and St. George, A. V.: M. Rec. **119**: 71, 1924.
4. Farmer, L.: J. Immunol. **36**: 37, 1939.
5. Karady, S., and Browne, J. S. L.: J. Immunol. **37**: 463, 1939.
6. (a) Knoll, A. F.: Proc. Soc. Exper. Biol. & Med. **45**: 606, 1940.
   (b) Alexander, H. L., and Bottom, D.: J. Immunol. **39**: 457, 1940.
   (c) Best, C. H., and McHenry, E. W.: J. A. M. A. **115**: 235, 1940.

7. Landsteiner, K.: The Specificity of Serological Reactions, Springfield, 1936, Charles C Thomas.
8. Clutton, R. F., Harington, C. R., and Yuill, M. E.: Biochem. J. **32**: 1119, 1938.
9. Butler, G. C., Harington, C. R., and Yuill, M. E.: Biochem. J. **34**: 838, 1940.
10. Hooker, S. B., and Boyd, W. C.: J. Immunol. **38**: 479, 1940.
11. Went, S., and Kesztyus, L.: Arch. f. exper. Path. u. Pharmakol. **193**: 609, 1939.
12. Strauss, M. B., and Spain, W. C.: Personal communication.
13. Coghill, R. D., Fell, N., Creighton, M., and Brown, G.: J. Immunol. **39**: 207, 1940.

DISCUSSION

DR. BEN Z. RAPPAPORT, Chicago.—I want to re-emphasize what Dr. Sheldon has not stressed sufficiently, that is, the preliminary nature of this report, the fact that the report is given very largely with the hope that it will stimulate further work from others in the same field.

While the work is not finished, nevertheless, it represents to those of us who have done some immunologic and some chemical work a tremendous amount to have been done in one year's time.

Dr. Sheldon is fortunate to have had such fine chemical and immunologic cooperation. While he has come to no final conclusion as to the manner of action of the substance he has used, I believe there are certain leads in his own data which might suggest a tentative opinion as to how the beneficial effect occurs. One can discount two of the three hypotheses, psychogenic effect, and the effect of histamine liberation, and consider the question of specific versus nonspecific foreign protein therapy. If it is a specific effect, as was shown by the animal experiments reported by Dr. Fell in a separate paper, then it is a specific effect not against histamine but against a conjugate of histamine and the protein that was used.

It is interesting in this connection that in his animal experimentation he had very similar results, no matter what protein was conjugated with histamine. The animal experiments, I understand, were similarly successful as measured by the precipitins produced whether casein or horse globulin was conjugated with histamine. This would indicate, since casein is a poor antigen, that the specific action was due to the haptene (histamine) or to the compound produced by the conjugation.

Now, if we compare the results on animals with those of the patients we find a distinct difference. We find that histamine-azo-casein does not give good results, while histamine-azo-globulin (horse globulin) does give good results. This difference in the results in animals and patients makes one question whether the beneficial effects were due to a specific foreign protein influence. The fact that the patients did not react to the casein histamine compound and did react to the horse serum histamine compound would indicate to me that he may have run across a good nonspecific foreign protein in the modified horse globulin. Unmodified horse globulin has seldom been used for its nonspecific effect because of the danger of urticaria. Here he has used safely horse serum globulin, which may be a more effective protein than typhoid vaccines or milk, or other foreign proteins. He has obtained good results in a certain group of cases, dermatologic cases especially, which are much more inclined, I believe, to give good results with nonspecific form protein therapy than hay fever and asthma.

However, this explanation does not conform to the fact that he does report good results in patients with hay fever. Despite the fact it was a mild season, the results are significant since they are as good as those he had with pollen therapy.

Very possibly, then, the mechanism of the effect of the globulin histamine compound is on a different mechanism than the pollen injections. The injection of pollen for instance, produces thermostabile antibodies. Does the protein conjugate used here produce the same antibodies?

Unfortunately, the entire field could not be covered in one year. In later work there may be a study of the effect of these injections on thermostabile antibodies, and that might help to establish the specificity or nonspecificity of this reaction.

This is a type of therapy that might be advised in a more extensive study with a smaller group of patients. It is the type of work that I believe will lead to greater advances by combining the efforts of the immunologist, the chemist, and the allergist.

Dr. Milton B. Cohen, Cleveland.—Ever since I became familiar with the experiments of Dr. Landsteiner in diazotizing antigens with simple chemical compounds, I have been hunting a chemist who would produce a histamine protein. It seems to me that we can accept, at least for a working hypothesis, the statement that the reactions which occur to any of the antigens which we normally use for skin testing or which produce symptoms in our patients are histologically and pharmacologically the same, and probably are reactions to histaminelike substances (H-substances), which may be histamine. I was very much encouraged, therefore, when I found that Dr. Fell had produced these histamine-azoprotein combinations. I understand from the report I have of a paper which Dr. Fell gave in Chicago, that, when animals were immunized with the horse serum-histamine combination and then sensitized, they could not be shocked.

I do not know whether he has done any work to show the presence of precipitins. If he has been able to get precipitins in any experimental animals, it would be important to know if the serum of the animal will precipitate both casein and horse-histamine azoprotein. If this occurred, the inference would be that the animal was sensitive to the histamine portion of the antigen molecule.

We have had some of these horse-histamine azoproteins for three months and have been injecting them in accordance with the technique described by Dr. Sheldon. I have been interested in what might be developing in the sera of these patients and am treating them to find out if they neutralize histamine.

In one provisional experiment we took the serum of the patient who was treated and mixed it with increasing dilutions of histamine. These mixtures, after letting them stand for a while, were tested by iontophoresis to determine the level at which a histamine reaction was obtained and, on the same individual's skin, determined the level at which a reaction to histamine alone could be obtained, i.e., the histamine threshold. In the one case there were between 10 and 20 multiples of protection.

I do not know whether that means anything or not. A great deal of work has to be done, but it seems to me this is very significant as an approach. Perhaps this approach which Dr. Sheldon and his group are bringing to us may be one of the answers to what our President said

to us this morning when he remarked that some chemists or endocrinologists might some day give us the exact answer to this problem.

If the premise which I made in the beginning is acceptable, that all allergic reactions are due to an H-substance, no matter what the antigen may be, and if we can produce a material which when injected into an individual will immunize him against the effects of H-substances, then we may be approaching what one old clinician said about Edwinil, "Give him a dose of Edwinil; it is a non-specific specific."

DR. WARREN T. VAUGHAN, Richmond.—As I interpret what I heard, we can pretty well eliminate the psychologic factor. We can consider nonspecific protein action in which the only specificity is in the material used, and I think we can still consider histamine effect.

Certainly one can get therapeutic results with doses of histamine that do not cause increased acid in the gastric secretion, so that I would not feel convinced that that test really ruled out the possibility of histamine effect. One has the possibility also of stimulation of histaminase.

I was thinking during the discussion that it is the type of problem that should be presented to a group like this, and to no more than to a group like this, and I was very pleased to hear Dr. Sheldon emphasize that this was a preliminary report, because we do not want to have a repetition of the histaminase episode. I should hate to see a pharmaceutical supply house start detailing a proposed but relatively untried product with the "detail men" quoting from a very carefully and honestly written paper, "Here is a new drug which can be used in any type of allergy. You don't have to do skin tests, and the results are just as good as in the specific treatment of hay fever, contact dermatitis, and so forth."

I think Dr. Sheldon's paper is fine. At the same time, I hope that the members of this organization can aid him in preventing premature use, before the value of the procedure has been established.

DR. ALBERT H. ROWE, Oakland, Calif.—During the last fourteen months histamine azoprotein has been administered to patients with various allergic manifestations which have been resistant to the accepted methods of allergic therapy. I felt that the real value of such a product could best be determined in such resistant patients.

Accordingly, it has been used in three such cases of perennial nasal allergy over periods of from three to six months without any benefit. No results were obtained with its use in four cases of bronchial asthma which had not been relieved by dietary, desensitization, and other accepted methods of treatment. Histamine azoprotein has also been given to four cases of atopic dermatitis which in my opinion are largely due to inhalant allergy. Duration of its administration has varied from three to six months. Two of these patients seem to have obtained some benefit. In one of them, improvement had already occurred with pollen therapy before the histamine azoprotein was administered. In the two other cases of atopic dermatitis no benefit was observed from the use of this product. There has been no opportunity to use it in cases of physical allergy.

Thus, after the use of histamine azoprotein over an extended period, I find no definite evidence of its therapeutic value in the treatment of allergic diseases, and its general use should not be encouraged unless, in the hands of many allergists over a period of several years, its value becomes unquestioned.

DR. SHELDON.—I should like to stress again that this report is a preliminary one and the problem is not yet answered. We realize there is a tremendous amount of work that needs to be done before any concrete evaluation can be definitely gained.

I appreciate all of the discussers' suggestions, including first, Dr. Rappaport's, concerning the study of thermostabile antibodies. I am sure that the control must be carried out with despeciated horse serum globulin. I have not done this because previous to this time it has not been known whether or not despeciated horse serum globulin would sensitize to horse serum.

I also appreciate the remarks of Dr. Cohen, and, with the President's permission, Dr. Fell is here and perhaps could answer the question Dr. Cohen has asked.

I also appreciate the remarks of Dr. Vaughan and Dr. Rowe. We should like to emphasize again that we do not believe that we have a panacea for the treatment of allergic diseases. What we have done is a little bit different from Dr. Rowe's work, in that we have attempted to select patients with known allergens in order that we might re-expose them to their own allergens after treatment. In this manner we had thought that we might better judge the effect of treatment.

A CRITICAL INTERPRETATION OF DATA ON THE INCIDENCE OF AIR-BORNE ALLERGENS

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ONE of the unsolved problems of inhalant allergy is that of accurate measurement of the varying amounts of antigenic material in the air. The answer to this question is of basic importance in the interpretation and evaluation of skin reactions and in the effective employment of air-borne allergens in treatment. The object of this paper is to appraise the accumulated results of atmospheric pollen and fungus spore research, to scrutinize the recently suggested methods for interpreting these findings and, if possible, to introduce simpler and more accurate concepts.

Seventy-five years ago Blackley[1] sensed the need of quantitative information on aerial pollen incidence. He sought diligently for a method of determining the number of pollen grains in a given volume of outdoor air. But after several fruitless experiments with various types of sampling apparatus, he discarded the volumetric method for the gravity slide method which has been widely used during the past two decades.

All agree that the data which may be obtained from uniformly exposed gravity slides by counting the particles caught on a unit slide area are highly satisfactory when dealing with the pollen of a single botanical species—common ragweed, for example. In an investigation covering species other than ragweed, the unit area figures would still be significant, provided all pollen particles under investigation were approximately the same size and had the same rate of fall. But since the widely differing size and surface characters of the various air-borne pollens cause appreciable differences in their rate of fall, it has long been believed that figures obtained by this method give a very inaccurate idea of the relative atmospheric contamination by pollens of various sizes. With this concept has come the assumption that all inaccuracies of statement can be avoided by determining the actual number of each kind of particle suspended in a given volume of outdoor air, as Blackley attempted to do.

Using Stokes' law, Scheppegrell[2] derived a formula and worked out tables for direct conversion of gravity slide figures into volumetric figures, i.e., the number of pollen grains of any species per cubic yard

From the Biological Laboratories of Abbott Laboratories, North Chicago.
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of air. His formula has been widely employed in the study of ragweed distribution but has never been strictly applied to statistics for other kinds of hay fever pollens. We have been content with unit slide areas (actual or calculated) ranging from 1 sq. cm. to 1 sq. ft., mostly with the accidentally adopted and admittedly awkward 1.8 sq. cm.

Unfortunately an error was introduced in Scheppegrell's formula of which we were not aware until Cocke[3] called our attention to it in 1937. Cocke proceeded with a corrected formula to revise Scheppegrell's conversion tables. Later he carried out a limited series of controlled experiments[4] with a volumetric apparatus and worked out correction factors for several kinds of pollen. These corrections are necessary because of the varying surface texture of different pollens. He found, for example, that for some unexplained reason the pollens of unnamed grasses 40 microns in diameter fall faster than their calculated speed, so that the theoretical figures must be reduced by 19 per cent. Other pollens whose theoretical figures must be reduced are hickory, walnut, willow, and paper mulberry.

Spiculated pollens and those with other gross surface irregularities have long been known to fall slower than Stokes' law allows. Cocke adds 32.2 per cent for ragweed, 7.5 per cent for sycamore, 8.6 per cent for oak, 14 per cent for plantain, and 56.2 per cent for pine. These figures are based on experiments in which the volumetric measuring apparatus was run for a total of only twenty-four hours—short runs of a few hours at varying times of day during May, June, and August. Only 50 cu. ft., less than 2 cu. yd., of air were handled. This it seems to me is hardly sufficient. Correction factors have not yet been supplied for the various grasses, including corn, nor are they available for the various *Amaranthaceae, Chenopodiaceae,* or *Artemisia.*

Cocke had no thought of introducing his unavoidably cumbersome volumetric device as anything more than a control for the indirect volumetric method as conceived by Scheppegrell. Briefly, Cocke counts 1 sq. cm. of a twenty-four-hour gravity slide, calculates the number of each kind of pollen per cubic yard of air, and states his results in the "number of pollen granules per cubic yard of air."

Obviously the practical difficulty in making use of this improved approach to volumetric counting is that a separate interpolation, as well as an arithmetical calculation, must be carried out for each figure representing each different kind of pollen on every slide counted. The revision of my own gravity figures accumulated from approximately 100,000 observations during the last sixteen years would involve more than a quarter of a million calculations.

As an illustration of the difficulties of application I shall cite a statewide pollen survey which is being conducted by the State Board of Health in Michigan. The statistics are being recorded in pollens of particular species "per cubic yard of air, according to Cocke's method."[5]

But on investigation I find that Cocke's corrections for various surface irregularities which account for aggregate errors as high as 50 per cent are not being used, not even for ragweed.

A glance at Cocke's tables is sufficient to show that our unit area figures for the smaller pollen granules, such as ragweed, fall far short of representing the true number of pollens per cubic yard of air, whereas our unit area figures for the larger pollen granules are somewhat near the actual number of pollens per cubic yard of air. He concludes, for example, that a ragweed count of 100 on the unit area 1.8 sq. cm. means that there were really 535 ragweed granules in a cubic yard of air, but that a count of 100 orchard grass granules represents only 99 granules per cubic yard of air. For particles smaller than ragweed pollen grains, the small fungus spores, for example, the discrepancy in our unit area figures is even more glaring. Certainly there is a great deal of satisfaction in being assured that the air really contains five times as much ragweed pollen as was heretofore supposed, even though we may still hesitate to change our method of statement and convert all old figures to the new standard.

It seems hardly worth while to convert our ragweed figures before we have all of the facts regarding accurate methods of calculating the atmospheric content of the other important hay fever pollens. We might do well to give heed to Vaughan's suggestion. He states that "the recording of atmospheric pollen concentration in terms of unit volumes of air gives the impression of scientific accuracy and appears to be the most desirable procedure. However, the accuracy of the method is probably more apparent than real, since there are too many variables."[6]

What are some of these variables that Vaughan fails to name? The most important one, I believe, is the mass of the pollen grain. Cocke may be correct as to the numerical volumetric pollen count but still be a mile from the goal sought by the allergist who compares the strength of antigens, at least in the laboratory and clinic, in milligrams per cubic centimeter. Does anyone honestly believe that we should count the pollen grains used in making pollen extract rather than weigh them? If so, we shall have to use about 200 Gm. of corn pollen to make 100 c.c. of corn pollen extract that will be comparable to a ragweed extract in which we use 3 Gm., for 1 Gm. of dry corn pollen contains only about 8 million granules as compared with 500 million to 1 Gm. of ragweed. Thus a few large pollen granules on a slide may be equal in antigen content to ten or fifty times as many granules of a smaller kind. So for allergists, a method of numerical statement of the pollen content of the air which ignores the mass factor may introduce more error than it seeks to cure. Why *count* the granules outdoors and *weigh* them when we bring them into the laboratory? To be consistent we should record atmospheric concentration in weight of pollen per volume of air.

TABLE I

VELOCITY OF FALL AND FORMULAS FOR COMPUTING CONCENTRATION PER CUBIC YARD FROM THE NUMBER OF SMOOTH POLLEN GRANULES PER UNIT AREA ON SLIDES EXPOSED FOR TWENTY-FOUR HOURS

DIAMETER (MICRONS)	VELOCITY OF FALL (FOOT-SECOND)	×* FOR	
		1 SQ. CM.	1.8 SQ. CM.
10	0.01	29.1	16.2
12	0.014	20.8	11.6
14	0.02	16.6	9.2
16	0.026	11.2	6.3
18	0.03	9.7	5.4
20	0.04	7.3	4.1
22	0.049	5.9	3.3
24	0.058	5.0	2.8
26	0.068	4.3	2.4
28	0.079	3.7	2.1
30	0.09	3.2	1.8
32	0.10	2.9	1.6
34	0.11	2.7	1.5
36	0.13	2.2	1.2
38	0.15	1.9	1.1
40	0.16	1.8	1.0
42	0.18	1.6	0.9
44	0.20	1.5	0.8
46	0.21	1.4	0.8
48	0.23	1.3	0.7
50	0.25	1.2	0.7
60	0.36	0.8	0.4
70	0.47	0.6	0.3
80	0.64	0.5	0.3

*Formula: $n = XN$.

N, Number of smooth pollen granules per unit area; n, concentration per cubic yard.

These are Cocke's calculations for velocity of fall and number of pollens per cubic yard of air calculated from the number on 1 sq. cm. To Cocke's formula is added the formula needed when the unit of counting is 1.8 sq. cm.

Let us revert to the comparison of ragweed and orchard grass figures by the gravity slide and volumetric methods. My unit area count revealed 100 granules of each per 1.8 sq. cm. Cocke's calculations gave 535 for ragweed and 99 for orchard grass. Assuming that these figures give the actual number of ragweed and orchard grass particles in the air, let us now calculate the mass of these particles and note the effect on our concept of the patient's contact with pollen antigen. A ragweed pollen granule 20 microns in diameter as compared with an orchard grass pollen granule 35 microns in diameter has less than one-fifth of the volume and probably one-fifth of the mass of the latter. So that the revised figures on a basis of probable antigenic units, pegging ragweed at 100, would stand ragweed, 100 and orchard grass, 107.

From this single illustration we might draw the conclusion that for pollen grains within a limited size range, 20 to 40 microns, our old gravity counts give as accurate ideas of the contacts of the sensitive patient as the more refined methods. Before doing so, we may well consider one more balancing factor. It may be that a single granule of corn pollen, when extracted, will yield as much antigen as sixty-four granules of ragweed pollen. But this does not prove that one granule

of corn pollen in one nostril and sixty-four granules of ragweed pollen in the other would cause equal irritation to a person who may be equally clinically sensitive to corn and ragweed antigens. Sixty-four small foci of infection may cause more temporary suffering than one large one.

In conclusion it should be reiterated that the end result can never be accurate as long as the sampling is open to question. The gravity method of sampling, on which we shall depend probably for a long time in large scale pollen surveys, is subject to large errors and can never approximate closely the actual exposure of the pollen-sensitive hay fever sufferer. But there is sufficient correlation between numerical incidence of pollens and the incidence of symptoms—onset, fluctuations, termination—to justify the work that has been done in sampling and counting. The measurement of incidence of suffering in inhalant allergy is even less accurate than our pollen statistics. So while admitting the need of a uniform method of stating atmospheric pollen and fungus spore pollution, we are not yet ready to adopt a laborious method which does not allow for factors that may later be proved to be of considerable importance.

SUMMARY

1. The gravity slide unit area method of investigation of the allergen content of the air is subject to much just criticism because, in using it as a substitute for volumetric measurement, we ignore the greatly varying rate of fall of particles of different sizes and surface characters.

2. Old errors in Scheppegrell's volumetric calculations from gravity slides have been corrected and checked by a direct volumetric device.

3. Numerical volumetric accuracy may now be had if we accept Cocke's calculations and amplify his work.

4. In achieving numerical accuracy, we may be overlooking a very important factor in the measurement of the antigen contacted by a sensitive human membrane—namely, the weight of the particles. The mass factor varies indirectly with the rate of fall factor, thus tending to cancel the importance of the latter.

5. The mass factor may be partially balanced by the multiple foci effect of small allergen particles.

CONCLUSION

A uniform method of determining the pollen and fungus spore content of the air is desirable, but no simple, accurate method has yet been proposed.

REFERENCES

1. Blackley, C. H.: Experimental Researches on the Cause and Nature of Catarrhus Aestivus (Hay Fever, or Hay Asthma), London, 1873, Baillière, Tindall & Cox.
2. Scheppegrell, Wm.: Hayfever and Hayfever Pollens, Arch. Int. Med. **19**: 959, 1917.
3. Cocke, E. C.: Calculating Pollen Concentration of the Air, J. ALLERGY **8**: 601, 1937.

4. Cocke, E. C.: A Method for Determining Pollen Concentration of the Air, J. ALLERGY **9**: 458, 1938.
5. Grigsby, B. H.: Personal communication.
6. Vaughan, W. T.: Practice of Allergy, St. Louis, 1939, The C. V. Mosby Company, p. 469.

DISCUSSION

DR. ABRAHAM COLMES, Boston.—I had the pleasure and privilege of reading Mr. Durham's paper yesterday. Two facts impressed me most, and these I should like to emphasize.

He stresses the inadequacy of the present method of estimating the pollen content in the air and to this we fully agree. Our own experiences in Boston had led us to believe that, for comparative studies, the Cocke method is better than the slide gravity method, although the latter is simple and adaptable and has given us a good deal of information.

I shall illustrate by slides, prepared during our Boston surveys, the correctness of these statements.

Another point he seemed to intimate is that there is a definite relationship between the height of the pollen curve and the amount of treatment needed, as though higher pollen curves necessitate a great deal more protection than do lower pollen curves. On this point I shall also agree with Mr. Durham, although sufficient variations occur to indicate that this does not always hold true.

Again, I shall illustrate the second point by slides.

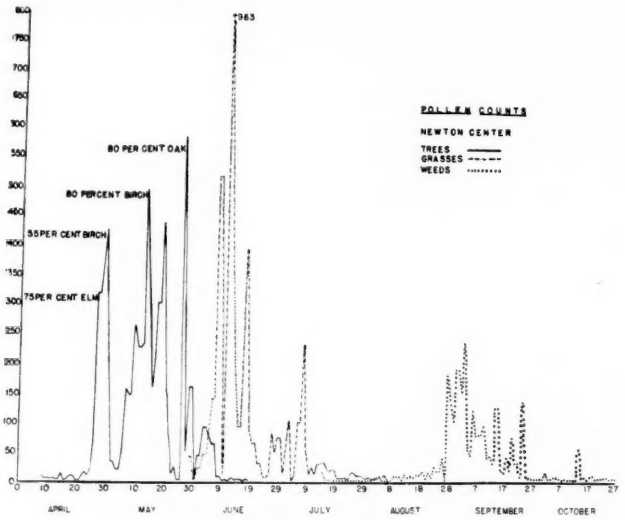

Fig. 1.

(Fig. 1.) In 1940, we carried out a pollen survey over six stations, three within the heart of Boston and three others within a radius of from 10 to 50 miles of Boston. The first slide represents the town of Newton, about 10 miles away, and on the right of the slide we have the ragweed pollen count, which is low; on the left we have the tree pollen count, which is extremely high considering its minor importance in relation to the pollens shed by the grasses and ragweed. In the center of the slide, we have the intermediary grass pollen curve. In this slide the counts were made by the gravity method.

(Fig. 2.) The second slide represents the same town of Newton where pollen counts were estimated by the Cocke method. Here the ragweed curve rises very high; the trees remain about the same; and the grasses show but little difference.

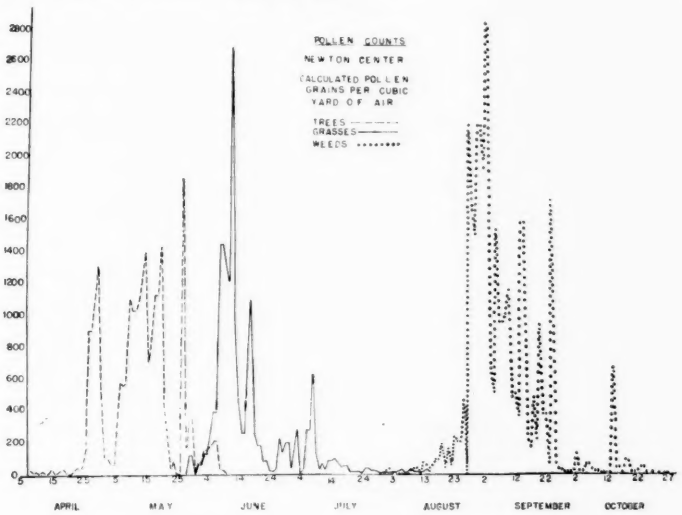

Fig. 2.

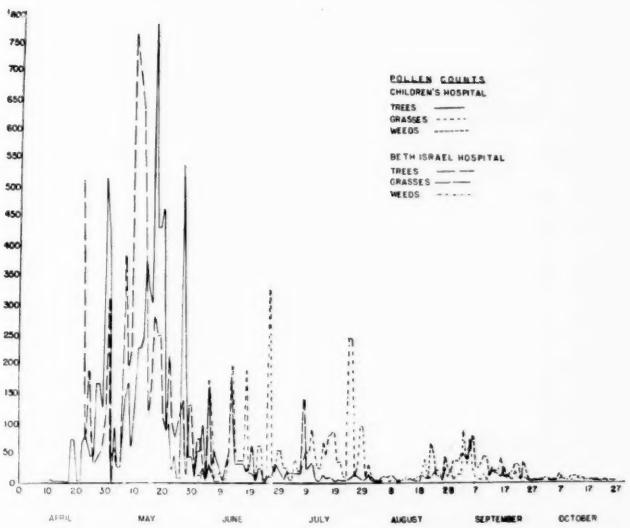

Fig. 3.

Thus, when we take into account the weight and circumference of the individual pollen granules, and the "rate of fall," our results seem more accurate.

Another error we have observed, even as others have, is the change in pollen counts when slides are exposed at different locations.

(Fig. 3.) The next slide represents two stations within 300 yards of one another: one at the Children's Hospital and the other at the Beth

Israel Hospital of Boston. Both slides were exposed at the same time of the day, for the same length of time, and yet the curves do not overlap. Evidently, if we are to have comparable results, we must have exposures in places which have the same relationship to the wind, vegetation, and other atmospheric factors.

All this adds to the errors of estimating the pollen grains in the air, and to the inaccuracies with which we are confronted. I do not believe, however, that such variations minimize in any way our efficiency in treating patients, and this point I should like to illustrate by presenting three additional slides.

During 1938 and since then we have handed out cards to patients in the clinic and to some private patients, and have asked them to record their experiences during the ragweed pollen season as being either good, fair, or bad.

Subsequently we correlated the "good," "fair," and "bad" days against the pollen curves. We found that, during "high pollen days," we have a great deal of suffering among patients and so for the group as a whole, this holds true.

When, however, the individual patient's experiences are recorded against the height of the pollen curves, the results are somewhat different. When the pollen count is low, many patients record bad days during that period. Others follow true to form and have a good many bad days during the height of the pollen curve, but what strikes us is the fact that a patient, who had a lot of discomfort before the pollens rose to a considerable height, lost his discomfort during the height of the pollen season, only to have more discomfort at the very end of the season when the count is on the decline.

Somebody will raise the question about mold sensitivity, and whether such sensitivity does not influence the course of the disease. I may state that, by skin tests and history, *Alternaria* was excluded as such possibility in these patients.

I do not believe that the pollen curve at all times is an index of the patient's need for treatment. If it were an index, it still would not tell us what the next season would be like, and, the severity of the pollen season not being known, we would not know how much pollen to give.

I am sure that Mr. Durham's paper is stimulating and interesting, and I should like to suggest that such attempts at investigating the various phases of pollinosis might lead us to a better understanding of the whole problem.

Dr. George Piness, Los Angeles.—We realized several years ago the importance of the type of work that Mr. Durham has done and so were led to attempt to find a method that would give us more detailed information. After the investigation of the various methods of dust collection and analysis used in industry, the impingement method was adopted.

We built an apparatus which consists of a coated slide moved by clockwork past a slit through which air is drawn at a known rate. Its construction and the results of its use will be reported later. With this machine, counts of the number of pollen grains or mold spores per unit of air volume can be made, independently of their rate of settling. Also, if the pollen concentration is high, accurate counts can be obtained in about fifteen minutes.

It was interesting to observe in this way the effect of activity in a previously quiet room; not only the dust but also the pollen count increased tremendously.

I do not wish to minimize the value of the gravity slide method, especially when the considerations Mr. Durham has proposed are kept in mind. Rather, our method has shown that the simpler method is adequate for everyday use.

Dr. Samuel M. Feinberg, Chicago.—How much air does that apparatus take in per unit of time?

Dr. Piness.—It draws 0.1 cubic foot per minute, 5.33 cubic yards per day, and draws it through the fine slit on top of the apparatus I showed you.

Dr. Theodore B. Bernstein, Chicago.—Is the apparatus which you have described portable?

Dr. Piness.—Yes, it is portable.

Mr. Durham.—Dr. Piness did not make it very clear that the slides exposed in his apparatus resemble electrocardiograms. They are really tracings of the amount of pollen in the air throughout the day. The usual twenty-four-hour gravity slide gives only the average atmospheric pollen concentration for the day but gives no idea of the variation at different hours of the day.

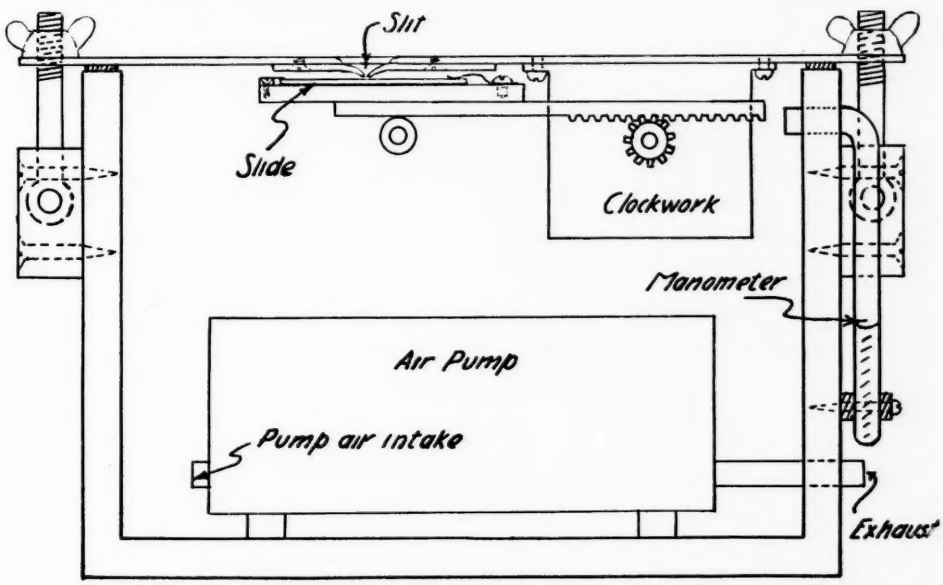

Fig. 4.—Dr. Piness' pollen counting apparatus. Simplified sectional view of the air-borne pollen sampling machine.

The standard method I have employed in counting all pollen slides exposed in my nation-wide studies is as follows: When using a 10-power ocular and 10-power objective, I count all pollens encountered in crossing the slide 5 times. The total distance traversed is thus 5 inches or 12.7 millimeters. The average width of the low-power field with the

lens combination I have mentioned is about 1.4 millimeters. Thus, the area covered is approximately 1.8 sq. cm.

Regarding the pollen statistics for Detroit, I can only say that the figures published in the Detroit papers are the result of counting some area other than the one I have used. Perhaps the one who furnishes these figures is using Cocke's method. Thus, the ragweed figures would be about 5 times as large as mine.

THE APPEARANCE OF POLLEN IN THE STOOL

E. L. MacQuiddy
Omaha, Neb.

IN JUNE, 1940, while the author was working in the laboratory of Dr. E. C. Faust at the Tulane University Department of Tropical Medicine, he was strongly impressed with the amount of unidentified pollen in stools in that locality. Two questions were raised. 1. Was this true in other localities? 2. By what route did the pollen enter the alimentary tract?

Theoretically, pollen in the stool can be carried there in food. There are very few foods, however, having pollen-bearing flowers at the time they are eaten. Discussions with dietitians and personal investigation found pollen-bearing flowers only in broccoli.

During the latter part of August and in early September, an attempt was made to discover if ragweed pollen could be found in the stools of people residing in Nebraska. Ragweed pollen is easily identified and is air borne; large quantities are in the air at that time of the year. Stools of normal persons, patients in private practice, and patients of the Allergy Clinic of the University of Nebraska College of Medicine were checked. Ragweed pollen was found in practically all stools examined (Fig. 1). It was felt that the only way this pollen could reach the alimentary tract was through the upper respiratory system.

During the month of January the author and three assistants attempted to determine the amount of ragweed pollen necessary to be ingested before it could be identified in the stool. Fifty milligrams of defatted pollen were mixed thoroughly with the noon meal. Charcoal tablets were taken simultaneously so that that portion of the stool containing the pollen could be easily detected. No pollen was found in these first stools. In order to avoid a possible accumulation of the pollen in the bowel, one week was allowed to elapse before a second dose of 75 mg. of ragweed pollen was ingested with the noon meal of Jan. 30, 1941. Again charcoal was administered as a marker. In the stools of Feb. 1, 1941, the pollen granules were identified in all of the four stools. Apparently the first dose of 50 mg. was an insufficient amount to be recognized.

To determine if any digestion of the pollen occurred in the intestinal tract, pollen stained with carmine was taken. Some of the stained pollen had previously been imbedded in paraffin, cut in 5 micron

Read before the Annual Meeting of the American Society for the Study of Allergy, Cleveland, Ohio, June 6, 1941.

sections, and found to be uniformly stained throughout. The pollen recovered in the stool showed very little trace of the stain. While not conclusive, it would seem that, as practically all stain was removed from the ingested pollen, some digestion had occurred while pollen was passing through the intestinal tract.

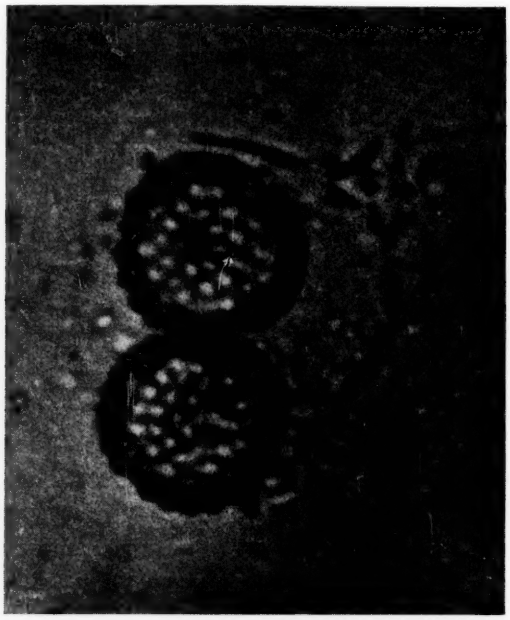

Fig. 1.—Ragweed pollen grains in the stool.

Pollen has been administered orally in the treatment of hay fever for a number of years. Curtis[1] reported that he used the tincture and fluid extract of ragweed blossoms and pollen in some eight or ten cases. He received his preparation too late to use preseasonally and began treatment two weeks after the commencement of the hay fever season. In all instances he claimed remarkable results. Scheppegrell,[2] in 1909, had patients snuff dried pollen in their noses two to three times daily. He made no mention of any gastrointestinal symptoms. Touart[3] used the method of administering pollen in salol-coated tablets and recorded no untoward effects. Black,[4] in 1927, believed that the stools of hay fever patients treated by the oral route contained an extract that would produce wheals in sensitized skins. In 1928,[5] the same author reported nausea, diarrhea, and abdominal distress in six patients treated orally. In the same year, Stier and Hollister[6] reported mild hay fever, mild asthma, diarrhea, irritated eyes, nausea, headache, stuffy nose, dizziness, and eczema when too large doses were administered orally. McGrew,[7] the same year, reported only increased intensity of hay fever symptoms when too much oral extract was taken. Rockwell[8] reported two cases of hives and a few cases of indefinite malaise. Bernstein and

his associates[9] reported marked gastrointestinal symptoms. Black[10] reported on two patients who complained of nausea and abdominal distress. Feinberg and his associates[11] reported that the oral ingestion of pollen in the doses used in their work was safe and that gastrointestinal reactions were frequent, troublesome, and sometimes severe, but not dangerous. Alperstein[12] reported gastrointestinal symptoms in twenty-eight patients, respiratory symptoms in two, and systemic symptoms of urticaria and general pruritus in three. The gastrointestinal symptoms occurred with a dose of 60,000 noon units; respiratory symptoms, with 30,000 and 60,000; and the systemic reactions, with 1,000, 2,000, and 60,000 units.

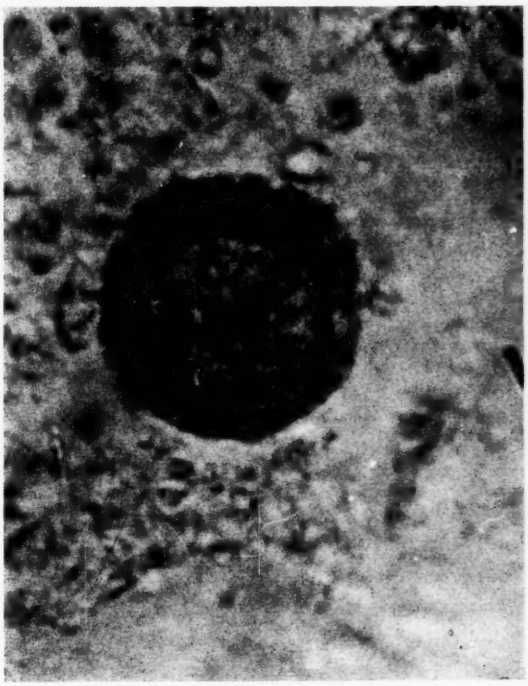

Fig. 2.

The question may be raised whether peptic digestion would destroy the activity of the extract of ragweed pollen. Bernstein and Kirsner[13] reported that they found this not to be true.

Since hay fever is a disturbance largely of the upper respiratory tract, the question whether materials ingested into the digestive tract influence upper respiratory symptoms also presents itself.

Rowe[14] mentioned food allergy as a cause of perennial hay fever and nasal congestion. Hansel[15] cited foods as inducing all types of allergy. Tuft[16] stated that foods and drugs may act as inhalant allergens and produce coryzal symptoms. Zeller,[17] while he could not demonstrate circulating antigens in patients treated orally, stated that there were

certain clinical symptoms which occurred with sufficient frequency to indicate absorption in some of the cases.

The finding of ragweed pollen in stools of normal and allergic patients during the hay fever season is not surprising when one considers the amount of saliva swallowed normally every day, and how, in the hay fever patient, the excessive secretion tends to wash accumulated pollens out of the nose.

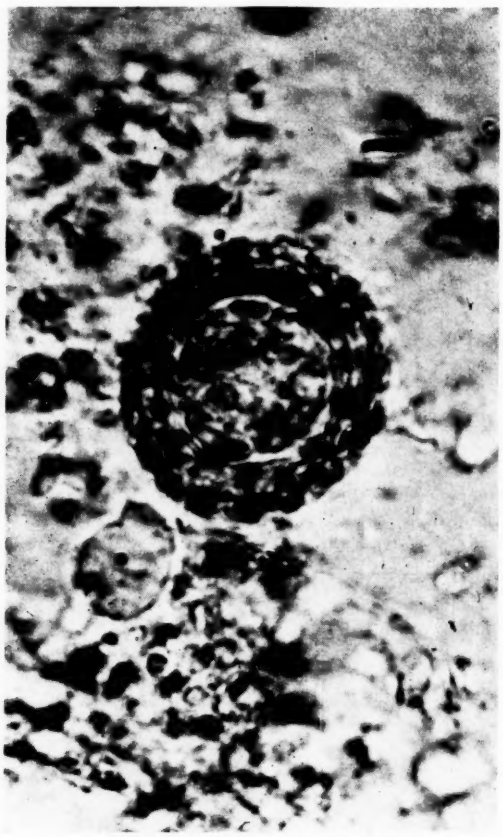

Fig. 3.

Whether or not a large part of the pollen inhaled finds its way into the digestive tract and whether part of the symptoms of hay fever may not be caused by ingested pollen as well as inhaled pollen are questions suggested by the study.

SUMMARY

1. Pollen in stools of normal and allergic patients during the hay fever seasons is reported.

2. So far as the author could determine, it was necessary to ingest 75 mg. of pollen before pollen grains in the stool could be recognized.

REFERENCES

1. Curtis, H. H.: The Immunizing Cure of Hay Fever, M. News **77**: 16, 1900.
2. Scheppegrell, W.: Hay Fever and Asthma, Philadelphia, 1922, Lea & Febiger, pp. 134-138.
3. Touart, M. D.: Hay Fever: Desensitization by Ingestion of Pollen Protein, M. Rec. **116**: 199, 1922.
4. Black, J. H.: The Oral Administration of Pollen, J. Lab. & Clin. Med. **12**: 1156, 1927.
5. Black, J. H.: The Oral Administration of Pollen; A Clinical Report, J. Lab. & Clin. Med. **13**: 709, 1928.
6. Stier, R. F. E., and Hollister, Guy: Desensitization by Oral Administration of Pollen Extracts, Northwest Med. **36**: 166, 1937.
7. McGrew, Captain G.: Time and Money Saved in the Treatment of Hay Fever, Mil. Surgeon **80**: 371, 1937.
8. Rockwell, G. E.: Clinical Results in the Prevention and Treatment of Hay Fever by Oral Administration of Pollens of the Grass and Ragweed Types, Ohio State M. J. **34**: 784, 1938.
9. Bernstein, T. B., and Feinberg, S. M.: Oral Ragweed Pollen Therapy, Arch. Int. Med. **62**: 297, 1938.
10. Black, J. H.: The Oral Administration of Ragweed Pollen, J. ALLERGY **10**: 156, 1939.
11. Feinberg, S. M., and others: Oral Pollen Therapy in Ragweed Pollinosis, J. A. M. A. **115**: 23, 1940.
12. Alperstein, B. B.: Oral vs. Parenteral Pollen Therapy, J. ALLERGY **11**: 498, 1940.
13. Bernstein, C., and Kirsner, J. D.: Oral Pollen Therapy, J. ALLERGY **8**: 221, 1937.
14. Rowe, Albert H.: Food Allergy, Philadelphia, 1931, Lea & Febiger.
15. Hansel, F. K.: Allergy of the Nose and Paranasal Sinuses, St. Louis, 1936, The C. V. Mosby Co.
16. Tuft, L.: Clinical Allergy, Philadelphia, 1937, W. B. Saunders Co.
17. Zeller, M.: Oral Ragweed Pollen Therapy, J. ALLERGY **10**: 579, 1939.

DISCUSSION

MR. O. C. DURHAM, North Chicago.—I am not able to answer Dr. MacQuiddy's major questions, but I can furnish a statistical background for his study. When he gave his experimental dose of 50 mg. of ragweed pollen in January, he gave approximately 25,000,000 ragweed pollen granules. A week later when he gave 75 mg. of ragweed pollen, the number of granules was approximately 37,500,000 ragweed pollen granules.

In the first part of Dr. MacQuiddy's paper he tells of finding ragweed pollen granules during the latter part of August and early September in almost all stools examined of residents of Nebraska. My figures for ragweed pollen for Omaha during the period mentioned show that the average number of pollens per cubic yard of air was 1,326 ragweed granules. An allowance of 15 cubic yards of air per patient per day would make the daily intake approximately 20,000 granules and for the whole season slightly over half a million granules.

Thus, the experimental dose of 50 mg. was about 50 times as much pollen as the average person in Omaha would be able to swallow during the whole season even if all inhaled pollens were swallowed. It seems reasonable that a fair proportion of 20,000 granules per day could be swallowed and recovered from the stools, but it seems improbable that a dose of 25,000,000 granules could disappear completely. The Omaha figures I have used are corrected by Cocke's method and are therefore most liberal.

DR. HARRY S. BERNTON, Washington, D. C.—I should like to ask Dr. MacQuiddy whether or not he has studied the allergenic activity of the pollen grains observed in the stools. It would be of interest to extract a specimen of stool with its contained pollen and test such an extract on a sensitized animal. The possible effects of the gastrointestinal juices on the allergenic activity of the pollen would then be determined.

DR. J. ALEXANDER CLARKE, JR., Philadelphia.—I should like to ask Dr. MacQuiddy if he has looked over the stools for any pollen other than ragweed. We do a great many pollen studies in the atmosphere trying to find out with what pollens these people come in contact, and it might be feasible, instead of going through a lot of complicated atmospheric pollen slides, to examine their stools and thus find out to which pollens they are exposed.

DR. FRANCIS M. RACKEMANN, Boston.—I should like to ask Dr. MacQuiddy whether he has observed any difference in the stools of normal people and the stools of hay fever patients as evidence of their ability to pass ragweed pollen through the gut.

DR. GEORGE L. WALDBOTT, Detroit.—In regard to Dr. MacQuiddy's theory that the pollen reaches the intestinal tract because it is swallowed with the sputum, I wonder if he has found any pollen grains surrounded by mucus.

DR. C. B. BOHNER, Indianapolis.—Dr. MacQuiddy used the oil-extracted material. I wonder if absorption would have been different had he used the material without extraction. After all, we are inhaling and ingesting pollens which contain oil.

I think we assume that pollen is rather quickly destroyed by absorption or by disintegration from evaporation, and yet botanists are able to determine the flora native to Indiana thousands of years ago, by the identification of specific pollens found in our muck beds. These pollens have been preserved in the acid media of the soil for hundreds of years. Is pollen destroyed at the end of the ragweed season, or do we have a ragweed problem even in the winter?

DR. MACQUIDDY.—In answer to Mr. Durham's criticism regarding the amount of pollen ingested and the amount of pollen that a person could breathe in, I do not have a good answer. It may be that our technique of finding the pollen in the stools was at fault. It may be that with more experience we can use amounts smaller than the ones used in these experiments.

In answer to the question whether or not there was any other pollen found, hemp pollen in July could be recognized in the stool. Hemp is not so characteristic a pollen as ragweed, and I chose ragweed for the subject of this paper because it was easily identified.

I could not see any difference between the passage of the pollen through the allergic and nonallergic intestinal tract. I was able to find pollen in practically all of the persons. Sometimes it was very difficult to find and we would have to make studies of several slides before we would find it. We had to rule out errors as far as possible and be sure that the pollen was not from the air. I recognize that as a possibility of error.

I cannot answer Dr. Waldbott's question about whether sputum was around the pollen grains found in the stool. I do not believe I can recognize it as such.

Then on this last question about the use of the defatted pollen, I realized that someone might raise the question. We have had difficulty in keeping our pollen without defatting it, so used the defatted pollen.

I am going to continue the work to see whether we can find pollen in the lungs. It has been already reported in the sputum. Scheppergrell, I think in 1906, reported finding of pollen in the sputum and in secretions from the sinuses. This is simply one more step.

I think all of us have been interested as to what becomes of pollen after it gets into the human system. I have been, for fifteen years, and I am glad to know now where part of it goes.

THE SPEED OF ACTION OF SO-CALLED "SLOW EPINEPHRINE" (IN OIL)

L. C. Bacon, M.D., M. Stickler, B.S., and R. W. Lamson, M.D.
Los Angeles, Calif.

OIL has been widely used as a medium for suspending or dissolving various hormones. It is generally accepted that such a medium causes slow absorption of the active ingredient. The efficacy of this method applied to epinephrine apparently was first pointed out early in 1930.[1] The method did not come into general use until recently when Keeney[2] and later Keeney and associates[3] prepared and used clinically a 1:500 suspension of powdered epinephrine base. Olive oil was first employed as a medium but later peanut oil was used. Shortly after this material became available for more general use, certain untoward reactions were noted and variously interpreted; thus Cohn[4] described rather severe manifestations which he had observed in four patients. He apparently interpreted these as evidence of underlying hypersensitiveness to peanut oil "as well as to side effects, the nature of which is not understood." More recently Dorwart[5] described a very severe response from the injection of 1.0 c.c. of epinephrine in oil which, according to the recommendations then current, had been preceded by an injection (0.3 c.c.) of aqueous epinephrine. We concur in his interpretation of the phenomena in that he does not believe that open vascular channels or sensitivity to the medium is the entire explanation. He warned against the use of such large doses as were originally recommended and felt that tolerance in his patient was low. Keeney[6] expressed the belief that the reaction observed by Dorwart was one directly referable to epinephrine. He emphasized that large gauge needles are conducive to rapid administration and possibly, therefore, to rapid absorption. He believed that a 23 or 25 gauge needle is more desirable than are needles of larger caliber. Because of the known variation in tolerance to epinephrine, he urged that the initial dose be chosen cautiously.

Insley and Segaloff[7] also felt that results such as Dorwart reported are typical of gross overdosage or of too rapid absorption. They, likewise, failed to ascribe these reactions to intravenous inoculation, and they believed that the initial dose should never exceed 0.5 c.c. although

From the Los Angeles County General Hospital and the Department of Medicine, University of Southern California Medical School.

Read at the Annual Meeting of the American Society for the Study of Allergy, Cleveland, June 2, 1941.

Much of the material used in this study was furnished through the courtesy of E. R. Squibb & Sons.

larger doses may be given subsequently after the patient's tolerance has been determined. The Council on Pharmacy and Chemistry,[8] in accepting this preparation for *New and Nonofficial Remedies,* recommended a still smaller quantity, 0.2 c.c., as the minimum dose, and agreed with the recommendation of Insley and Segaloff that the initial dose for adults should never exceed 0.5 c.c. Subsequent to Keeney's studies it was suggested[9] that epinephrine 1:500 be suspended in gelatin solution rather than in oil. Kalmon[10] compared the two products and apparently preferred the oily mixture. The study we are reporting herein concerns only the latter preparation.

Our study of the literature mentioned above indicates that there are several factors which may have influenced these earlier results. In many instances the patients were hospitalized, a factor which in itself often affords marked relief. Secondly, results from the first few doses of any remedy used in the treatment of asthma may be more dramatic than those from subsequent doses. The relatively large doses—as compared to the amounts of the aqueous preparation more commonly used—might make the oil preparation appear superior. Perhaps the most significant factor is the amount of optimism or psychotherapy administered with a new preparation. Many physicians consciously or otherwise thus enhance the action of any remedy which they employ.

In our study, all thirty-six patients were ambulatory although their symptoms were so severe that they did not respond to the usual remedies. There were twenty males and sixteen females ranging in age from 10 to 72 years. Thirteen had had asthma for less than ten years, and twenty-three, for ten years or more. Fourteen patients were hypersensitive; twenty showed no sensitivities; and two were not tested. The patients were not informed as to the nature of the material nor of anticipated favorable or unfavorable results. The injections were given by a physician, and the patients were under constant observation by him for at least thirty to forty-five minutes, unless a physiologic response was detected in a shorter interval. Each patient was required to keep a record of the exact time when symptoms recurred and some estimation of their severity in order that we might make a comparison with the patient's condition before treatment.

The ampoule of epinephrine in oil was shaken vigorously for several minutes—the duration and intensity of shaking exceeding any preparation the patient himself would employ—to insure a well-emulsified suspension. A 24″ by 5/8″ needle was used, and it, as well as the syringe, was sterile and dry. If several injections were to be made in rapid succession from the contents of the syringe, they were spaced by no greater interval than that which was required to change needles. If longer intervals were necessary, the suspension was transferred to a small, sterile, dry, glass vial fitted with a rubber or synthetic rubber cap. Before each

aliquot was removed, the material was again vigorously shaken. The consistency of results, even over a period of a year or more for each patient, suggests that equal volumes contained identical potency. It may be emphasized that we employed all orthodox procedures in an attempt to prevent the material from entering vascular channels. In no instance was there a suggestion of such an accident. We attempted to introduce the oil under (or at the edge of a bundle) rather than into the body of the muscle. Pain and discomfort were practically nonexistent even though maximum doses of the oil suspension were employed.

The work was begun early in 1939 at a time when the larger doses were in vogue. We were soon convinced that these were not only unnecessary but, in many instances, undesirable; on a few occasions we have observed unpleasant reactions from the minimum dose of 0.2 c.c. In several instances these reactions occurred in patients who previously had tolerated and responded favorably to larger doses. None of these was interpreted by us as being a manifestation of true sensitivity to the vehicle or to the epinephrine. In several patients the initial response to this preparation, and incidentally to the aqueous mixture, was an actual intensification of cough and dyspnea. Since marked relief followed in another two to three minutes, the response was not interpreted as being an idiosyncrasy.

The following incidents exemplify several of the points we have made. A Mexican male, aged 28 years, experienced severe itching of his nose and also substernal burning after the use of the oil preparation. At times these symptoms became quite distressing, and the vehicle was suspected. After he had been without the oil preparation for one week, he was given 0.7 c.c. of aqueous epinephrine (1:1,000). In a few minutes he experienced the same kind of nasal and substernal irritation which had followed the use of the oil mixture. With the aqueous as with the oil preparation he likewise obtained relief. This seems to exclude a sensitivity to peanut oil. A 61-year-old male patient often, but not inevitably, experienced severe prostration following minimal doses of either preparation. A 59-year-old woman experienced a euphoric state which occasionally began within two minutes after the injection of the minimum dose (0.2 c.c.) of the oil suspension. While her reaction time was consistently short, another patient might require approximately ten minutes and still another fifteen or twenty minutes before any response is evident. The time factor can be but little reduced even when huge doses are employed; it seems to be a relatively fixed characteristic for each particular patient. Even though this period be as long as twenty minutes, the maximum for the majority of patients, it hardly justifies designating the material as "slow epinephrine." Such variations in the rate of response to aqueous preparations are quite common.

Our findings duplicate the observations made independently by a considerable number of patients who used adrenalin or other epinephrine in oil. One young woman who occasionally used this medication to prevent or control urticaria experienced no systemic manifestation from doses of 1.0 c.c. or more. The drug, however, had a pharmacologic effect.

Early in our study it became evident that many of these patients did not require a preliminary dose of aqueous epinephrine, an observation which we made without knowledge of the report by Murphy and Jones,[11] who treated nine patients with intractable chronic asthma with slow epinephrine and commented that "it was apparently as rapid and efficient for the relief of attacks as is ordinary epinephrine hydrochloride." Incidentally, they also anticipated another of our findings, namely, that the use of the oil preparation does not necessarily mean a reduction in the actual amount required as compared to that used with the ordinary solutions of epinephrine. Our investigation was therefore concerned with the use of the oil preparation alone and with an effort to determine the effectiveness of minimum doses in the ambulatory rather than in the hospitalized patient.

Since but two of a total of thirty-six patients were under 20 years of age, they did not furnish a basis for conclusions relative to the value of this preparation in treating younger patients. Although each patient was unusually small for his age, slightly more than the minimum dose always was required for effective relief. It has been our experience that children with asthma do not respond as favorably as adults and that almost inevitably they require amounts of medication out of all proportion to their age and weight.

Table I shows the results of the entire series of 128 injections. The average time elapsing before any response was noted represents quite accurately the latent period for the particular dose in relation to the severity of symptoms. The duration of relief following the use of this agent has received considerable emphasis in earlier studies, and, if one depends entirely upon these reports, especially those dealing with hospital patients, it would appear that the benefit is more prolonged than it is after comparable amounts of the aqueous preparation. Other studies as well as our own failed to support such claims. Prolonged relief, except when more than a few hours, probably represents a temporary remission rather than a continuing supply of the drug. Hence, those patients who had no symptoms for several days after one injection are not included in the averages. In approximately 33 per cent of patients receiving 0.3 to 0.5 c.c. a definite reduction of symptoms occurred and persisted for periods of one to seven days; in a few instances the reduction was complete. Since the interval between injections was usually a week or more, the effects noted probably do not represent a summation of the new and any previous injections. Although it cannot

TABLE I

RESULTS OF 128 INJECTIONS OF EPINEPHRINE IN OIL GIVEN TO 36 PATIENTS

SEX	0.2 c.c.			0.3 c.c.			0.5 c.c.			0.6 c.c. TO 0.8 c.c.			1.0 c.c. TO 1.5 c.c.		
	TOTAL NO. OF PATIENTS	RESPONSE (AV.)		TOTAL NO. OF PATIENTS	RESPONSE (AV.)		TOTAL NO. OF PATIENTS	RESPONSE (AV.)		TOTAL NO. OF PATIENTS	RESPONSE (AV.)		TOTAL NO. OF PATIENTS	RESPONSE (AV.)	
		BEGAN (MIN.)	PERSISTED (HOURS)		BEGAN (MIN.)	PERSISTED (HOURS)		BEGAN (MIN.)	PERSISTED (HOURS)		BEGAN (MIN.)	PERSISTED (HOURS)		BEGAN (MIN.)	PERSISTED (HOURS)
Female	6	8	6	7	8	13	9	7	29	5	9	24	4	10	10
Male	7	17	5	6	10	17	13	13	21	10	15	24	8	12	9
Failures	1 Male			1 Female			2 Males						1 Male		
Summary	Response within 15 minutes after 9 of a total of 14 injections			Response within 10 minutes after 16 of a total of 26 injections			Response within 10 minutes after 23 of the 44 injections (within 20 minutes after all but 4 injections)			Response within 10 minutes in 11 (in 15 minutes in 23) of the 33 injections			Response within 10 minutes after 15 of the 25 injections		

be shown in Table I, we noted many times that a certain amount might be effective on numerous occasions, while on the next visit the patient might require five to six times the previous quantity to afford relief. Such results demonstrate the need for great flexibility of dosage, a need which cannot best be met so long as the preparation is supplied only in 1 c.c. ampoules, where the tendency is to use the entire dose or to waste the remainder—a very expensive procedure.

It should be noted that the male patients, probably because of their known higher threshold against most drugs, required a slightly longer interval before they detected any effects from these injections. The larger doses apparently were not much more rapid in their action than smaller ones. This should be accepted only with reservations since the larger doses were not employed unless the symptoms were more severe. When these larger doses were employed, the period of relief rarely exceeded twenty-four hours and was usually less than ten. This also is due to the intractable nature of the symptoms. If any therapeutic agent fails under such extreme conditions, it is suggested that the physician should administer some other agent rather than continue to increase the dose of the ineffective one.

SUMMARY

1. Evidence is presented to indicate that epinephrine in oil is not slow in its action.

2. The time between its administration and its pharmacologic effect compares favorably with that for equivalent doses of the aqueous preparation.

3. Contrary to general belief, small doses of epinephrine in oil frequently are effective.

REFERENCES

1. Insley, S. W.: Control of Immediate Shock Following Hypodermic Medication, Preliminary Report, J. A. M. A. **94**: 765, 1930.
2. Keeney, E. L.: A Slowly Absorbed Epinephrine Preparation, Bull. Johns Hopkins Hosp. **62**: 227, 1938.
3. Keeney, E. L., Pierce, J. A., and Gay, L. N.: Epinephrine in Oil; a New, Slowly Absorbed Epinephrine Preparation, Arch. Int. Med. **63**: 119, 1939.
   Keeney, E. L.: Epinephrine in Oil. Its Effectiveness in the Symptomatic Treatment of Bronchial Asthma, Am. J. M. Sc. **198**: 815, 1939.
4. Cohn, J.: Unusual Reactions to Slow Epinephrine, J. ALLERGY **10**: 417, 1939.
5. Dorwart, F. G.: Slow Epinephrine—An Experience, J. A. M. A. **114**: 647, 1940.
6. Keeney, E. L.: Reactions of Epinephrine in Oil; Correspondence, J. A. M. A. **114**: 1098, 1940.
7. Insley, S. W., and Segaloff, A.: Systemic Reaction to Slow Epinephrine; Correspondence, J. A. M. A. **114**: 1826, 1940.
8. Council on Pharmacy and Chemistry: New and Nonofficial Remedies, Suspension of Epinephrine in Oil, 1:500, J. A. M. A. **116**: 1221, 1941.
9. Reel, F. C.: Epinephrine: Gelatin Mixture, West Virginia M. J. **36**: 126, 1940.
10. Kalmon, M.: Clinical Comparisons of Epinephrine in Oil and Epinephrine in Gelatin, J. ALLERGY **12**: 55, 1940.
11. Murphy, J. A., and Jones, C. A.: Slow Epinephrine in Treatment of Chronic Asthma, J. ALLERGY **10**: 215, 1939.

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DISCUSSION

DR. OSCAR SWINEFORD, JR., Charlottesville, Va.—This paper makes two points. It points out the facts (1) that small doses of adrenalin in oil are effective and (2) that they are effective promptly, within a matter of a few minutes.

I have little to add to what has been said. I want to emphasize one thing, which was mentioned, and which I suppose all of you know. The ampoules of adrenalin in oil contain the crystalline adrenalin which, when put in the icebox, settles out in a small lump at the bottom of the ampoule. If you do not mix the material thoroughly before you administer it, you are likely to get the symptoms of adrenalin overdosage.

Facts that have been noted in this paper had been stated previously by Murphy and Jones, who called attention to the fact that the adrenalin in oil has essentially the same time element for action that a 1:1,000 aqueous solution has, and Keeny, the originator of this term "adrenalin in oil," pointed out that small doses can be effectual.

I think the method of administering antispasmodic drugs in enteric-coated capsules has to a large measure done away with the necessity for using adrenalin in oil, which is a troublesome procedure. The patient should take five minutes or so to mix the material properly in order to administer it, and it is hard to get it through a needle that is comfortable to use for injection.

I simply want to re-emphasize that, in the absence of proper emulsification of the drug, you are likely to get the reactions which have been quoted in the literature, namely, the adrenalin shock and occasionally pulmonary edema. Also sloughing locally, headache, and nervousness have been reported; subarachnoid hemorrhage has also been reported.

SEASONAL AND GEOGRAPHIC INFLUENCES ON FOOD ALLERGY

ALBERT H. ROWE, M.D.
OAKLAND, CALIF.

FOOD allergy in certain patients decreases in the summer and increases during the winter. In California the beneficial effect begins in mid-March to mid-May and ends in mid-August to mid-November, on the average from one equinox to the other. Severe immediate reactions from food allergy apparently are not affected or, if so, to a lesser degree than are the chronic manifestations which are periodically exaggerated in more or less severe attacks with intervening partial or complete relief due to desensitization or refractoriness.

This seasonal effect on food allergy which has been observed for approximately fifteen years, was reported in 1931,[1] and discussed further in 1937.[2] Bronchial asthma due to food allergy, especially in children and young adults, often becomes increasingly persistent with periodic exaggerations in the winter months and is affected frequently by this seasonal influence. There are a few records of allergic migraine and recurrent sick headache recurring from September or October to April or May and absent or much decreased in the summer. Nasal allergy due to foods also may be improved in the summer, and activated in the fall, winter, and early spring, producing so-called "sinusitis" or "head colds" which often last for several weeks. These seasonal effects have not been observed in gastrointestinal allergy from food. In all cases where it had been decided that these seasonal influences on food allergy existed, the symptoms were nearly or entirely absent during the winter when the allergenic foods were eliminated. Many of these patients with bronchial asthma, moreover, either had no nasal or respiratory infections during the winter or had no asthma when such infections occurred, providing there was strict adherence to the elimination diet. Thus, the increasing frequency of, and susceptibility to, respiratory infections from September to April in the general population cannot explain either the actual allergic attacks or any secondary influence in their production. Atopic dermatitis due to food allergy, especially when localized on the hands and flexures, is definitely exaggerated in certain cases in the winter, improving or disappearing in the summer. This type of dermatitis can well be called winter eczema and, of course, can be due also to inhalants and contactants encountered more frequently or

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only in the winter. The dermatitis in our patients referred to in this article was relieved almost completely or entirely by the exclusion of allergenic foods in the winter without any environmental changes.

Most of our patients have lived near San Francisco Bay, the Pacific Ocean, or in the coastal valleys. A few have lived in the large inland valleys and in states adjoining California. A few patients report this seasonal effect in the eastern as well as in the central states, and histories of relief in the summer in Europe have been obtained. Just how widespread this seasonal influence on food allergy is should be observed by allergists in this and other countries.

Possible explanations of these seasonal effects need consideration. There is increasing humidity during the winter and early spring in northern California. However, when the increased susceptibility recurs from August to November, the humidity is lower than at any other time of year. In fact, in June and July on the Pacific coast, there is much fog during the time when food allergy often is least active. Petersen has suggested that the tendency to increased blood alkalinity, which develops during certain summers more than during others, might affect muscle spasm and that the lower pH values in the winter might increase susceptibility to allergy. However, the administration of alkali or acid for several weeks has not produced uniform effects on allergic reactivity. Van Leeuwen[3] in 1932 reported this tendency for allergic symptoms to appear or increase from August 15 to October. After making experimental studies, he concluded that ionization of air, variations in air pressure, and humidity were not explanatory. In the San Francisco Bay area, especially, changes in barometric pressures cannot explain these seasonal effects, since very slight changes in pressure occur in the summer, fall, and early winter months. Moreover, in this area there are few sudden changes from a warm to a cold front in the weather and the slower developing warm front is infrequent. Thus, the acknowledged effect of frontal weather on tissue metabolism and disease cannot explain the increasing susceptibility to allergic reactions in the early fall to spring months in our territory. The regular recurrence of the asthmatic attacks due to food allergy, moreover, could hardly be influenced by the irregularly occurring solar eruptions which cause electrical magnetic disturbances on the earth. The most logical reason for the benefit of the summer months seems to be the increased available solar radiation, especially when there is more outdoor life. As this radiation decreases in the late summer and fall months, especially in the winter even when the weather is still moderate and warm and outdoor activity is not restricted, the susceptibility to reactions from food allergy increases. If such radiant energy is responsible for the summer improvement, it is not due to the ultraviolet radiation which can be produced artificially by quartz light. The continued use of such

therapy with additional viosterol does not make these food-sensitive persons less susceptible to their causative food allergens in the winter months.

Recognition of this seasonal effect on food allergy has helped us in the diagnosis and the later successful control of the patient's symptoms. A history of such seasonal variations in symptoms suggests food allergy with the necessity of skin testing and the subsequent use of elimination diets[3] in most patients. As foods are added to the diet of the symptom-free patient, moreover, patients are forewarned that foods may be tolerated in the summer which will produce symptoms with increasing frequency from September until December and January, decreasing gradually to April or May. If future observations in other areas indicate similar seasonal effects on food allergy, the same help from the clinical viewpoint generally will be of value.

GEOGRAPHIC EFFECTS ON FOOD ALLERGY

Bronchial asthma, allergic bronchitis, and nasal allergy due to foods are often relieved when the patient goes 5 to 10 miles away from the ocean or bay shores over the first range of hills in northern California even though the allergenic foods are still being eaten. This activating effect on food allergy by the marine atmosphere also has been noted on a few patients with allergic headache, gastrointestinal allergy, and cutaneous edema. One of our patients had swelling of the eyelids, nausea, and mild headache from fresh fruits and egg in the bay area and not inland. The relief that many patients with nasal and bronchial allergy, so-called chronic sinusitis, recurrent headaches, gastrointestinal symptoms and other chronic symptoms experience in the inland, mountainous and desert areas of California, the southwest and the high plateau areas of the Rockies may be due in part to the etiological role of food allergy.

In bronchial asthma, especially, and also in nasal allergy due to food sensitization, this maritime influence is most frequent. Many patients know that ocean climates precipitate their symptoms. The history of a 12-year-old boy with bronchial asthma is typical. When he was at home at Bolinas on the ocean shore, recurrent attacks had been present for five years from September to May with slight or no attacks from June to mid-August. In Healdsburg, however, about 20 miles inland over the coast range his attacks were absent even in the winter without any change of diet. For the last three years, he has been on an elimination diet from which milk only is excluded at present. He has been entirely free from all attacks throughout the year even though he has lived on the ocean shore where much summer fog and wind and much winter and spring rain are the rule. Thus, in this case the beneficial effect of summer months already discussed was present along with the detrimental effect of maritime areas, especially at other times of year.

Another patient, a 10-year-old boy, gave a history of recurrent bronchial asthma when near the ocean. For five years the family had lived in Colusa on the Sacramento River some 100 miles from the ocean and bay. Here he was entirely free of asthma. Wheezing, however, immediately developed on inhaling the bay air near Vallejo and increased to definite asthma as he rode 10 miles along the bay shore to Berkeley. In a few hours after returning to Colusa, his asthma would disappear entirely. For the last nine months, he has been on a cereal-free elimination diet. At the end of the first month of this diet he had no wheezing until he had been in Berkeley for five hours. At the end of the second month wheezing developed within two days, and in three months he remained in Berkeley in the rainy month of January with no asthma for one week. This development of bronchial symptoms and less often of nasal symptoms with the first inhalation of the maritime air has been observed in many of our patients. Other patients must remain a few hours, a day, or longer before this maritime effect occurs.

Our conclusions regarding the geographic influences on food asthma are based on patients where asthma is controlled definitely in the ocean and bay areas when allergenic foods are eliminated from their diets. We have fully recognized the possible effect of the varying inhalants in different regions, and no symptoms due to inhalant allergy formed the basis of our conclusions.

Speculation concerning the cause of this maritime effect on food allergy, especially when bronchial asthma or nasal allergy results, is important. It has been suggested that the increased humidity in the bay and ocean areas, especially in foggy weather, may affect the sympathetic and vascular tissues producing mucous membrane edema, congestion, and smooth muscle spasm. Although the humidity in our territory is never high except in very rainy weather, it is usually greater than that away from the bay, especially in the areas over the first hills of the coast range. Thus, a change in humidity is usually experienced in coming from the inland to the maritime area. Favoring humidity as a cause is the increased benefit of the desert areas of southern California, of the high mountains, and the southwest. Against humidity, however, is the experience of patients such as that of the boy from Colusa whose symptoms are absent in the winter even though he lives on the Sacramento River where there is more rain and land fog than in the bay area. Elevation and changes in barometric pressure, moreover, play no fundamental influence since the below sea level areas of the Saltine Sea and Death Valley are as beneficial as are the higher inland regions.

There is also the possibility that some characteristic of the air near the ocean water may be responsible. The minerals of sea water from evaporated mist blown inland from the ocean are found in decreasing amounts 5 to 8 miles inland. Such content of the air is evident by the

smell of the ocean. That an activating effect on allergic reactions might occur from this mineral content of the air is problematical.

Whatever the explanation, it is certain that the effect is due to the so-called secondary causes of allergy in which odors, smoke, weather and temperature changes, nervousness, and fatigue belong. They activate underlying allergies and fail to produce symptoms in most patients when the causative allergies are recognized and under control.

Our observations have been confined to patients of the Pacific coast, especially in the San Francisco Bay area. Whether other maritime areas or the Great Lakes area affect food allergy in a similar way has not been discussed in the literature. The marine asthma reported by Jimenez-Diaz and his co-workers[5] in Spain was relieved by inland climates, and this indicates that food allergy may be a possible cause.

The history of this bad effect of maritime climate and the benefit of inland climates on food allergy, especially on bronchial and nasal allergy, is helpful in suggesting food allergy as one possible cause of the symptoms. Such possible geographic influences should always be noted and recorded in routine history taking.

SUMMARY

1. Food allergy in certain patients, especially when it produces bronchial asthma, nasal allergy, atopic dermatitis, and at times gastrointestinal, cutaneous, and cerebral symptoms, becomes less active or even absent in certain patients in the summer months, increasing in its activity to a maximum during December and January.

2. Food allergy, especially when it produces bronchial asthma and less often nasal allergy, may be activated in maritime areas near the ocean or the bay as observed in northern California and may disappear or decrease in its activity in inland areas even 5 to 10 miles away from the salt water.

3. These seasonal and geographic effects on food allergy as observed in northern California, especially in the San Francisco Bay area, may both occur in varying degrees in the same patient or either one may act alone without any evident effect of the other.

4. All food-sensitive patients are not affected by these influences. Those with chronic food allergy, especially with bronchial and to a lesser degree nasal allergy, are most susceptible.

REFERENCES

1. Rowe, A. H.: Food Allergy, Philadelphia, 1931, Lea & Febiger.
2. Rowe, A. H.: Clinical Allergy, Philadelphia, 1937, Lea & Febiger.
3. Rowe, A. H.: Elimination Diets and the Patient's Allergies, Philadelphia, 1941, Lea & Febiger.
4. (a) Van Leeuwen, W. S.: L'Influence du sol et du climat sur l'asthme, Paris, 1932, Masson & Cie.

(b) Idem: Klimatisch-geographische Medizin. Asthma, Bronchitis und Schnupfen in Zusammenhand mit der Jahrsegeit, München. med. Wchnschr. **79:** 583, 1932.
(c) Idem: Studien ueber die physiologische Wirkung des Föhns, München. med. Wchnschr. **8:** 293, 1932.
(d) Idem: Klimatisch-geographische Medizin. Studien über die physiologische Medizin, München. med. Wchnschr. **79:** 293, 1932.
5. Jimenez-Diaz, C., Sanchez Cuenca, B., and Puig, J.: Climatic Asthma, J. ALLERGY **3:** 396, 1932.

DISCUSSION

DR. FRANCIS M. RACKEMANN, Boston.—I think this paper has elements of considerable importance. The difficulty I find is that the observations and the suggestions made are so very hard to control. The little boy had trouble at the shore and went 10 miles inland to find relief. That is typical enough, and all of us have just such cases as that, and the fact gives rise to my insistence upon taking a decent history. I would explain the little boy as being sensitive to some foreign dust substance which he found on the shore and from which he escaped by going over the hills into the country; and I think it would be very difficult to prove or disprove that he was sensitive to such a dust. One way of doing it would be to take his bedding from the country and have him move it to the shore, and then find that he remained quite comfortable at the shore!

I have a number of such cases that have been relieved in just such a simple manner.

Of course, the trouble could be pollen. I have heard that the climate and flora of California change abruptly from one place to another, and in a relatively short distance. It would be easy to believe there is some pollen at the shore which does not occur up in the hills. The point is as I say, that the control of these observations would seem to me very difficult, chiefly because of the dust factor.

On the other hand, I try to be broadminded and I can believe there are still other factors in allergy which play a part. Certainly it is true that all our so-called winter asthmas—and also the atopic asthmas and the chronic perennial vasomotor rhinitides, so-called—on the whole are better off in summer than they are in winter. As Dr. Rowe says, those changes could depend upon humidity, upon solar radiation, or ultraviolet light, upon warmth, or simply on the fact that the patient is outdoors more and that, in turn, means exercise, with greatly improved physical well-being, and, incidentally, a better balance between the total food intake and the total energy output. Whether any, all, or some combination of those factors plays a part, I do not know, but I think that it is helpful and important to hear such a paper as this if only to make us think of factors other than foods and dusts. Dr. Rowe adds a little to my own conception that: "All is not allergy that wheezes."

DR. GEORGE PINESS, Los Angeles.—It seems to me that most of us have a different explanation for the question of the relation of sensitivity to foods to seasonal sensitivity. Those of us who have found that hay fever patients did not get well despite active treatment with specific pollen antigen, and then later found that these same patients were sensitive to foods, discover that, with their elimination, our results were improved.

On the other hand, patients who were sensitive to foods and moved to another environment were not always relieved. Those who were relieved

when apparently sensitive to foods and foods alone, on subsequent study, perhaps by someone else, were often found to be sensitive to something within their immediate environment, so that the very patient whom Dr. Rowe mentions, who went 10 miles inland and found relief, might have moved next door and been as well or better.

So I cannot be convinced that food sensitivity changes with the seasons, particularly when patients are sensitive to foods that are utilized in the daily diet throughout the year.

These two observations sufficiently explain the seasonal variation of symptoms in food-sensitive hay fever patients, for they demonstrate that, when two factors are involved, one seasonal and one perennial (pollen sensitivity and food sensitivity), the seasonal factor may appear to have a perennial character and the perennial factor a seasonal one.

DR. HOMER E. PRINCE.—To me, practicing in a sea coast town, this paper was very interesting, for I am confronted with this problem all the time.

We have a number of patients in Houston and in the Gulf Coast area of Texas generally, who seem to fall into Dr. Rowe's description. We have no mountain range for them to go across, but, when those patients do not get well, their parents frequently (I am referring particularly to children) take them to San Antonio, and frequently by the time they get 25 miles away from the coastal area, they clear up. However, sometimes in the winter they do not do so well, particularly during wet seasons in San Antonio.

To me the whole thing is not a matter of food; it is not altogether a matter of environment, either, but a matter of unfavorable influences, lowering the threshold of clinical difficulty. In better weather, better environment, they do better than when everything is against them.

DR. I. S. KAHN, San Antonio.—I shall limit my discussion of Dr. Rowe's paper to the cutaneous manifestations (urticaria, atopic dermatitis) in allergic individuals, usually with asthma, which have cleared under climatic change. I do not believe that any one of us has seen a large number of these cases. The one or two instances I have encountered have been the direct reverse of Dr. Rowe's experience. In my cases, where the pollen asthma has been complicated by cutaneous manifestations, confirmed by positive skin tests for foods and where a rigid diet was required in the heavy pollen environment of San Antonio, residence in the summer on the Texas sea coast with a greatly lessened pollen contact, and with alleviation of respiratory symptoms, has permitted the eating of any food desired without cutaneous symptoms.

House dust precautions, comparatively easy in our mild climate, I often find essential for the complete relief of apparent cutaneous allergy due to foods. Many of us have produced or made worse, eczema and urticaria, by overdosage with pollen or house dust extracts. Hence, I am inclined to agree with Dr. Piness and Dr. Rackemann in the probable elimination of a contact in these cases of Dr. Rowe, probably something connected with bedding, upholstery, or some house dust factor.

DR. ROWE.—My article distinctly states that the climatic and seasonal influences discussed were only in food-sensitive patients. Inhalant allergy was entirely absent in many of these patients such as those whose histories are summarized in the article. When food allergy was not controlled, symptoms were present in the bay region or on the coast, where-

as, with the elimination of allergenic foods, no symptoms occurred in the same houses near the bay or coast without any change in the environment whatsoever. Other cases in which these effects on food allergy have been observed during the last fifteen years, of course, have also been subject to inhalant allergies of various types which have necessitated proper therapy. But in these cases also the same seasonal and geographic influences on their concomitant food allergy have also been noted. As stated in the article, all patients with food allergy have not been affected by these geographic and seasonal changes.

It is interesting that Dr. Prince has observed similar geographic effects on his patients in Houston. Dr. Cline of Seattle has also noted such benefit from living over the mountains from Seattle. It will be of interest if allergists in other maritime areas will study patients suffering with food allergy to determine if the geographic and seasonal effects I have observed in northern California occur in other areas. Of great importance is the study of the explanation of these influences.

Original Articles

THE SENSITIVITY OF MAN TO HUMAN DANDER, WITH PARTICULAR REFERENCE TO ECZEMA (ALLERGIC DERMATITIS)

STANLEY F. HAMPTON, M.D., ST. LOUIS, MO., AND
ROBERT A. COOKE, M.D., NEW YORK, N. Y.

IN THE early years of the development of allergy, from about 1912 to 1920, when skin testing for sensitivity was an important procedure, attention was directed mainly to a search for substances which might give skin reactions. Animal danders and sera as well as pollens and foods were first used for testing because of the clinical observations that had established their casual relationship. Nothing was more natural than that human dander should also be tried. Many allergists of that time made human dander extracts and discarded them as unimportant because of the large number of suggestive skin reactions on normal as well as on allergic persons. These reactions were due, as we now know, to the crudeness of the extracts.

In 1922 Van Leeuwen and his co-workers[1] published the first paper on human dander and reported that this extract reacted on direct skin test in most allergic persons and that it was diagnostic of the "allergic state." A subsequent paper[2] advised dialyzation to remove a "nonspecific irritant" which caused skin reactions in normal persons. In 1926 these authors[3] reported that 90 per cent of all allergic persons reacted to human dander extract but nonallergic persons never reacted.

In 1929 Van Leeuwen and Van Niekerk[4] emphasized the fact that human dander extract and extracts of three layers of skin each produced skin reactions in "almost" all allergic human beings. Feinberg and his co-workers[5] confirmed Van Leeuwen's findings in a report of twenty-five cases of asthma and of nine normal control subjects.

Murphy and Cobe[6] made a study of fifty allergic and fifty nonallergic persons. Eighty per cent of the allergic group and fifty-five per cent of the nonallergic group gave a three-plus or greater reaction to a 1:1,000 dilution of human dander extract. In a dilution of 1:10,000,000, approximately 20 per cent of the allergic group still showed reactivity while the nonallergic group were negative. These authors made

From the Department of Allergy, The Roosevelt Hospital, New York City, N. Y.
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the significant observation that the reactions with human dander extract and with histamine in various dilutions were "almost identical."

Keller[7] reported that all of twenty-one cases of "spatexsudative ekzematoid" were positive by skin test to dialyzed human dander extract. None of eleven "exsudative ekzematoid" cases and none of five "flexual neurodermite" cases gave positive skin tests. Two of thirteen "ekzema," one of eight "dermatitis," and one of twenty-three "seborrhoisches ekzematoid" cases were positive. Only twenty-four of 252 cases of other skin disorders (scabies, syphilis, acne, psoriasis, trichophytid, etc.) were sensitive to human dander.

In a recent study[8] from this clinic, Dale tests on guinea pigs sensitized to house dust extract were found to be positive to a number of the common inhalants, including human dander. Because the Dale reactions showed human dander to be one of the antigens in house dust, generally considered as an important factor in certain allergies but whose active principle is still unknown, we decided to restudy skin reactions to human dander. Existing work gives conflicting results, probably due to the fact that a histamine-like substance was present in the extracts of some of the experimenters. The present study soon indicated the importance of eczema* to which particular reference will be made.

PREPARATION OF THE EXTRACT

Human dander and hair combings from normal scalps were washed with ether and then extracted overnight at 7° C. with an alkaline-saline fluid. The extract was then dialyzed against running water for twenty-four hours, and 0.5 per cent NaCl, 0.35 per cent $NaHCO_3$, and 0.4 per cent phenol were added. The extract was filtered through a Seitz filter, tested for sterility, and standardized on the basis of its protein nitrogen content.† These extracts were the ones used also in the study[8] previously referred to and were shown not to contain any histamine-like substance.

DIRECT SKIN TESTS

These tests were done on patients with eczema, with allergic diseases other than eczema, and with dermatoses other than eczema and on non-allergic persons giving negative clinical and family histories of allergy.

Testing directly with a human dander extract containing 10 or 100 units per cubic centimeter usually failed to give significant reactions in patients who had given marked reactions when tested with extracts containing 1,000 and 5,000 units per cubic centimeter and whose sera had transferred the sensitivity to normal skin. Even an extract of

*The authors recommend the term "allergic dermatitis" rather than "eczema" to denote this state. Because of common usage and for the sake of clarity, however, the term "eczema" will be used in this paper.

†One protein nitrogen unit = 0.00001 mg. of protein nitrogen. Hereafter in this presentation "unit" refers to the protein nitrogen unit.

1,000 units occasionally failed to produce more than a slight reaction although moderate or marked reactions were obtained with 5,000 units. In all tests 0.01 to 0.02 c.c. of extract was injected intradermally.

An extract containing 5,000 units per cubic centimeter was therefore chosen for routine testing, although the 1,000 unit extract was frequently used. Results were recorded as "negative" when no wheal or erythema developed, "slight" when the wheal and erythema were less than 1 cm. in diameter, "moderate" when the erythema and the wheal were 1 cm. or more in diameter, but without pseudopods, and "marked" when a larger wheal with definite pseudopods and erythema developed.

TABLE I

RESULTS OF DIRECT SKIN TESTS WITH HUMAN DANDER EXTRACT CONTAINING 5,000 UNITS PER CUBIC CENTIMETER

GROUP	NEGATIVE	SLIGHT	MODERATE	MARKED	TOTAL
Eczema	2	3	9	14	28
Other allergies	41	39	11	2	93
Normal	50	25	0	0	75
Total	93	67	20	16	196

TABLE II

RESULTS OF DIRECT SKIN TESTS WITH HUMAN DANDER EXTRACT CONTAINING 5,000 UNITS PER CUBIC CENTIMETER ON NINETY-THREE PATIENTS WITH ALLERGIES OTHER THAN ECZEMA

DIAGNOSIS	NEGATIVE	SLIGHT	MODERATE	MARKED	TOTAL
Asthma	16	12	7	2	37
Hay fever	18	21	7	2	48
Vasomotor rhinitis	7	7	4	1	19
Urticaria	6	2	1	0	9
Gastrointestinal disorder	2	0	0	0	2
Total	49	42	19	5	115

In several of the cases of eczema, the extent of the active skin lesion or lichenification of the skin prevented direct testing and necessitated study by passive transfer.

Twenty-three of twenty-eight patients with eczema showed moderate or marked skin reactions. Only thirteen of the ninety-three patients with "other allergies" and none of seventy-five normal persons showed more than a slight reaction to human dander on direct skin test. Two of the five eczema patients who failed to show at least a moderate skin reaction had only mild patchy eczematoid lesions on the hands. Infants did not in general react as strongly on direct skin test as did older persons with eczema. In fact, no marked reactions were obtained in the infant group.

When patients with "other allergies" were classified according to clinical diagnoses, there was no evidence that such patients reacted to human dander extract as frequently as those in the eczema groups. A

TABLE III

SUMMARY OF ECZEMA CASES

CASE	AGE	OTHER ALLERGIES	DURATION OF ECZEMA	DIRECT TESTS WITH HUMAN DANDER EXTRACT		PASSIVE TRANSFER TESTS WITH HUMAN DANDER EXTRACT (5,000 UNITS PER C.C.) DILUTIONS OF SERA							
				1,000 UNITS PER C.C.	5,000 UNITS PER C.C.	UNDILUTED	1:10	1:30	1:100	1:300	1:1,000	1:3,000	1:10,000
Jb.	21 yr.	Hay fever	3.5 yr.	Moderate		++	++	+	+	+	0		
Dys.	9 yr.	Hay fever, asthma and vasomotor rhinitis	9 yr.	Moderate	Moderate	+++	++	+	±	0	0		
Sde.	30 yr.	Hay fever and asthma	6 yr.	Slight	Moderate	+++	++	++	+	0	0		
Sbn.	17 yr.	Hay fever	1 yr.		Marked	+++	++	+	+	±	0		
Rte.	2.5 yr.	Asthma	2 yr.		Moderate	++	+	+	+	0	0		
Rgi.	27 yr.		27 yr.		Marked	+++	++	++	++	+	0		
Ars.	10 yr.		2 yr.	Moderate	Marked	++	+	±	0	0	0		
Dfk.	3 yr.		2.5 yr.		Moderate	+++	++	++	++	±	0		
Awt.	43 yr.		30 yr.			+++	++	++	++	±	0		
Mfk.	10 yr.	Asthma and vasomotor rhinitis	10 yr.		Moderate	+++	++	+	+	0	0		
Ceh.	19 yr.		5 yr.	Marked	Marked	++	+	+	0	0	0		
Tsn.	16 yr.	Asthma	Life		Marked	+++	+++	+++	++	+	+	+	0
Rbl.	1.5 yr.	Asthma	1 yr.	Slight	Moderate	++	+	+	0	0	0		

Vpr.	10 mo.		9 mo.			+	0	0	0	0	0		
Hcy.	24 yr.	Asthma and hay fever	4 yr.	Marked	Marked	+++	++	++	+	0	0		
Afo.	16 yr.	Asthma	Life	Marked	Marked	++++	++++	+++	+++	++	+	0	
Fvd.	2.5 yr.		2 yr.		Moderate	++	++	+	0	0	0		
Fsh.	6 mo.		6 mo.	Slight	Moderate	++	++	+	0	0	0		
Emc.	39 yr.	Asthma, hay fever, and urticaria	20 yr.	Moderate	Moderate	+++	+++	+++	++	±	0		
Jhe.	2 yr.		1 yr.			+	±	0	0	0	0		
Edy.	12 yr.		12 yr.	Marked	Marked	+++	+++	++	+	0	0		
Mlz.	6 mo.		4 mo.			++	+	±	0	0	0		
Ren.	5 yr.	Asthma and angioneurotic edema	5 yr.	Slight	Slight	++	+	0	0	0	0		
Hbd.	24 yr.		2 yr.	Moderate		+++	++	++	++	+	0		
Mar.	44 yr.		30 yr.		Marked	++	+	0	0	0	0		
Emy.	23 yr.		Life		Marked	+++	++	++	++	++	+	0	
Aks.	28 yr.		24 yr.	Marked	Marked	+++	++	++	++	+	0		
Gsr.	22 yr.		Life	Marked	Marked	+++	+++	++	++	+	+	0	
Cbd.	12 yr.		8 yr.	Marked	Marked	++++	++++	+++	+++	++	0	0	
Hgy.	34 yr.		Life	Marked	Marked	+++	+++	+++	+++	++	+	±	0
Lck.	24 yr.	Asthma and vasomotor rhinitis	5 mo.	Negative	Negative	0	0	0	0	0	0		
Cbn.	3 yr.	Asthma	3 yr.	Slight	Slight	0	0	0	0	0	0		
Jsk.	10 yr.		1 yr.	Negative	Negative	0	0	0	0	0	0		

TABLE IV

SUMMARY OF CASES OF ALLERGIES OTHER THAN ECZEMA

CASE	AGE (YR.)	DIAGNOSES	PAST HISTORY	TESTS WITH HUMAN DANDER EXTRACT						
				SKIN TESTS		PASSIVE TRANSFER DILUTION TESTS WITH 5,000 UNITS PER C.C.				
				1,000 UNITS PER C.C.	5,000 UNITS PER C.C.	CONCENTRATED	1:10	1:30	1:100	1:300
Rys.	32	Hay fever		Slight	Moderate	++	+	0	0	0
Dsn.	29	Asthma and hay fever	Eczema (age 13-17 yr.)	Slight	Moderate	+	±	0	0	0
Wen.	34	Asthma and hay fever	Eczema (age 7-21 yr.)	Slight	Moderate	++	+	0	0	0
Asn.	38	Hay fever	Fungus infection of skin (20 yr.)	Slight	Moderate	+	±	0	0	0
Abr.	39	Asthma and vasomotor rhinitis		Moderate	Moderate	++	++	+	0	0
Rtn.	10	Hay fever	Psoriasis (7 yr.)	Slight	Marked	+++	++	++	+	0
Jbd.	43	Hay fever		Moderate	Moderate	++	++	+	0	0
Ehl.	25	Hay fever			Moderate	+	+	0	0	0
Msn.	50	Asthma and hay fever		Slight	Moderate	++	++	+	+	0
Fas.	15	Vasomotor rhinitis		Slight	Marked	+++	++	+	0	0

number of the patients had multiple clinical allergies and have therefore been counted more than once (Table II).

PASSIVE TRANSFER TESTS

Passive transfer tests were carried out in the skin of test subjects who had shown negative intradermal tests to human dander extract (5,000 units per cubic centimeter). Serum studies were made on all patients who had given moderate or marked skin reactions to human dander, on some allergic patients, not skin tested, and on a number of allergic and normal persons who had shown negative or slight direct skin tests. Serum sites were prepared with 0.05 c.c. of serum and tested forty-eight hours later with 0.025 c.c. of human dander extract (5,000 units per cubic centimeter).

The sera of thirty of thirty-three patients with eczema transferred the skin sensitivity to normal skin, thus indicating sensitizing antibodies for human dander extract. The sera of only ten of ninety patients with "other allergies" (including cases of asthma, hay fever, vasomotor rhinitis, and urticaria) and none of ten normal control sera gave positive reaction on passive transfer. The sera of four of five eczema infants and eight sera from eczema patients who were not themselves tested gave positive passive transfer reactions, while twenty-one sera of patients with other allergies who were not skin tested failed to show skin-sensitizing antibodies.

In no instance did sera from patients showing negative results by direct skin tests contain skin-sensitizing antibodies for human dander extract. Two sera from eczema patients (3 and 5 years of age) who had only slight reactions by direct skin test were positive on passive transfer. All other sera from allergic or normal persons giving slight skin reactions failed to sensitize normal skin. All of twenty-three sera of eczema patients showing moderate or marked skin reactions (by direct testing) were positive in passive transfer tests. In the group of other allergies eight of the eleven sera of patients showing moderate skin reactions and both of the two sera showing marked skin reactions were positive in passive transfer tests. The duration of the eczema and concomitant allergies, when present, are shown in Table III. The diagnoses for the ten patients with other allergies whose sera gave positive transfer are shown in Table IV. Two of these patients (Dsn., Wen.) gave past histories of skin lesions suggesting eczema; another (Asn.) had a chronic fungus infection of the thigh for twelve years; and a fourth patient (Rtn.) had had intermittent psoriasis for seven years. The remaining six patients had no history of skin lesions.

DILUTION TESTS

All sera that reacted in passive transfer sites were studied to determine the quantity of skin-sensitizing antibodies present. One-tenth

cubic centimeter of various saline dilutions of the respective serum was used to prepare skin sites, and the sites were tested forty-eight hours later with 0.025 c.c. of human dander extract containing 5,000 units per cubic centimeter.

Table III shows the reaction in passive transfer sites made with dilutions of serum from each eczema case. In most instances large reactions were obtained. Twenty-one of the thirty sera were still positive when diluted 100 times with physiologic saline, and five were positive at a dilution of 1:1,000. No definite correlation could be drawn between the amount of antibody present in the serum and the duration of the eczema, although all five sera that reacted in dilution of 1:1,000 were from patients who had had eczema all their lives. In the other allergy groups positive reactions were rarely obtained in dilutions greater than 1:30 (Table IV).

NEUTRALIZATION TESTS

Using the passive transfer technique, neutralization experiments were carried out on nineteen of the sera from eczema patients. Equal quantities of serum and increasing strengths of human dander extract were mixed in test tubes, and 0.1 c.c. of each mixture was used to prepare skin sites in test subjects. These sites were tested forty-eight hours later with 0.025 c.c. of the extract containing 1,000 or 5,000 units per cubic centimeter.

It was found that human dander extract neutralized human dander sensitive serum so poorly that it was frequently necessary to use a 1:10 dilution of a serum in order to demonstrate an end point of neutralization (Table V).

Cross Neutralization With Human Dander and House Dust Extracts.—Simultaneously with the above, using sera that sensitized passive transfer sites to human dander and house dust extract, we studied the ability of these extracts to cross neutralize the sensitizing serum. Three typical experiments are given. In serum Gsr., human dander extract in high concentrations neutralized against house dust extract. In other sera, partial neutralization occurred. With one exception (Gsr.) this was demonstrated only when the sera were diluted 1:10 and concentrated human dander extract was used. This one exception was a patient whose serum showed only a small reaction to dust in passive transfer sites. Likewise, house dust extract in high concentrations did neutralize to a degree against human dander extract. Complete neutralization was demonstrated in five instances when 1:10 serum dilutions were used. Three of the thirty sera (eczema group) that contained skin-sensitizing antibodies for human dander extract failed to sensitize skin to house dust extract in a 1:10 dilution. In these three instances 5,000 or 10,000 units of dust extract had neutralized the sera in 1:10 dilutions to test with human dander extract (serum Hel.). Since these three sera, in the dilution used, did not react to house dust,

TABLE V

NEUTRALIZATION OF HUMAN DANDER SENSITIZING SERUM WITH HUMAN DANDER AND DUST EXTRACTS

SITE NO.	SERUM MIXED WITH	PROTEIN NITROGEN (UNITS PER C.C.)	REACTION ON TEST 48 HOURS LATER WITH 5,000 UNITS PER C.C.	
			HUMAN DANDER	DUST
		Serum Rgi. Concentrated		
1	Human dander	10	++	++++
2	Human dander	100	+	++++
3	Human dander	1,000	±	++++
4	Human dander	5,000	0	++++
5	Dust	10	+++	++++
6	Dust	100	++	+++
7	Dust	1,000	+	++
8	Dust	5,000	+	+
9	Saline		+++	++++
		Serum Gsr. Concentrated		
1	Human dander	100	++	+
2	Human dander	1,000	+	+
3	Human dander	10,000	0	0
4	Dust	100	+++	+
5	Dust	1,000	+++	+
6	Dust	10,000	+++	+
7	Saline		+++	+
		Serum Hcl. 1:10 Dilution		
1	Human dander	10	++	0
2	Human dander	100	++	0
3	Human dander	1,000	+	0
4	Human dander	5,000	0	0
5	Dust	10	++	0
6	Dust	100	++	0
7	Dust	1,000	++	0
8	Dust	5,000	0	0
9	Saline		++	0

it seems likely that there was human dander in house dust extract and the human dander was responsible for the neutralization. The presence of human dander in house dust extract as demonstrated by the Dale test has been reported.[8] Likewise, human dander extract would be expected to contain traces of house dust, in view of the manner in which the dander was obtained. This may explain the occasional neutralization by human dander extract to test with dust extract. Furthermore, the reaction of the serum sites to dust may, in some cases, be due to the human dander in dust extract.

This study demonstrates that house dust extract neutralizes house dust-sensitizing serum very poorly, and even high concentrations of the extract frequently fail to neutralize completely.

It is a common observation that patients with eczema usually react to a skin test with house dust extract. Just as it is suggested from these experiments that human dander and dust extracts are contaminated with one another, the frequency with which patients with eczema react to house dust may be due to the presence of human dander in dust.

PRECIPITIN TESTS

Precipitin tests were carried out with seven sera of patients with eczema showing skin reactions in high dilutions, one serum of a non-eczematous allergic person (serum contained skin-sensitizing antibodies), and one serum from a person not sensitive to human dander extract. All sera failed to show precipitins by the Lancefield technique.

HEAT LABILITY

The sera of seven patients with eczema showing skin reactions in high dilution to human dander extract were heated to 56° C. for four hours. These heated sera failed to sensitize normal skin whereas the respective unheated control sites reacted when tested with human dander extract.

DERMATOSES

(CASES FROM DERMATOLOGY CLINIC)

In order to determine whether the skin sensitivity to human dander extract occurred in dermatoses other than eczema, eighty patients were tested with human dander extract (5,000 units per cubic centimeter) by the same technique, in the Dermatologic Clinic at The Roosevelt Hospital. The results of these tests support the findings of Keller.[7] Two of eleven cases diagnosed "contact dermatitis" gave moderate skin reactions, and the serum of one of these patients was positive on passive transfer test. One of five patients with acne gave a moderate reaction, and the serum of this patient was positive on passive transfer. Two cases of exfoliative dermatitis, one based on arsenical poisoning and the other of unknown etiology, gave a negative skin test to human

TABLE VI

RESULTS OF DIRECT SKIN TESTS WITH HUMAN DANDER EXTRACT CONTAINING 5,000 UNITS PER CUBIC CENTIMETER ON DERMATOSES PATIENTS SEEN IN THE DERMATOLOGY CLINIC AT THE ROOSEVELT HOSPITAL

DIAGNOSIS	NO. OF CASES	DEGREE AND NO. OF REACTIONS			
		NEGATIVE	SLIGHT	MODERATE	MARKED
Tinea	17	11	6	0	0
Seborrhea	10	10	0	0	0
Contact dermatitis	11	8	1	2	0
Impetigo	5	5	0	0	0
Dermatitis venenata	5	3	2	0	0
Acne	5	2	2	1	0
Psoriasis	6	5	1	0	0
Varicose eczema	4	3	1	0	0
Scabies	2	2	0	0	0
Exfoliative dermatitis	2	2	0	0	0
Herpes zoster	2	2	0	0	0
Prurigo nodularis	2	1	1	0	0
Prurigo aestivalis	2	2	0	0	0
Pityriasis rosea	1	1	0	0	0
Lupus vulgaris	1	1	0	0	0
Eczema	5	0	1	3	1
Total	80	58	15	6	1

dander extract, even after six weeks of marked desquamation. There were five eczema patients examined, and all were positive by direct skin and passive transfer tests.

RELATIONSHIP OF HUMAN DANDER SENSITIVITY TO OTHER ANIMAL DANDER SENSITIVITIES

The suggestion has been made that persons reactive to other animal dander extracts might also be reactive to human dander extract. This was based on the hypothesis that the keratin in various animal danders was identical. It has been shown by Vickery and his co-workers,[9, 10] Block,[11] and Wilkerson[12] that keratins in the skin of various animals are chemically similar. Wilkerson and Tulane,[13] however, have shown that keratin from human skin contains less cystine and more methionine than that of other animal skins. Furthermore, Pillermer, Ecker, and Wells[14] have shown that, although keratins of various epidermal tissues are chemically similar, there is a species specificity as shown by precipitin tests.

In order to determine whether human dander sensitivity as determined by the skin test on man was specific and unrelated to other animal danders, direct and passive transfer skin tests were carried out on a number of patients (Table VII).

Many eczema patients are reactive to various animal danders, and their sera contain skin-sensitizing antibodies. Furthermore, these patients often show sensitivity to many inhalants other than danders and to many foods. It is apparent, however, that the active principle in human dander extract is not a substance common to animal danders.

DISCUSSION

Human dander extract, prepared from human dandruff and rendered histamine free, has been shown to be reactive in certain human skins, and certain human sera have been shown to possess a capacity to sensitize normal human skin to this extract.

Thirty of thirty-three patients with eczema were reactive to human dander extract and possessed sera containing skin-sensitizing antibodies for it; "other allergies" rarely and normal persons do not act similarly. Therefore, one is led to believe there is a relation between eczema and dander sensitivity. Whether this sensitiveness is a cause or a result of the eczematous lesions is not determined in this study. Two of the ten noneczema allergic persons (Table IV) whose sera contained skin-sensitizing antibodies for human dander gave definite past histories of eczema, suggesting that the phenomenon is a result of eczema. Furthermore, the failure to obtain such past histories in the remaining eight cases does not necessarily rule out the presence of eczema in infancy or early childhood.

TABLE VII

RESULTS OF DIRECT AND PASSIVE TRANSFER SKIN TESTS WITH HUMAN AND ANIMAL DANDER EXTRACTS CONTAINING 5,000 UNITS PER CUBIC CENTIMETER

CASE	SKIN TESTS	DANDER				
		HUMAN	HORSE	DOG	CAT	RABBIT
Emy.	Direct	++++	0	0	0	0
	P. T.*	++++	0	0	0	0
Wen.	Direct	++	0	0	0	0
	P. T.	++	0	0	0	0
Ars.	Direct	++++				
	P. T.	++	0	0	0	0
Mar.	Direct	++++	0	0	0	0
	P. T.	++	0	0	0	0
Rbl.	Direct					
	P. T.	++	0	0	0	0
Fsh.	Direct	++				
	P. T.	++	0	0	0	0
Sbn.	Direct	++++	0	±	±	0
	P. T.	+++	0	0	0	0
Hey.	Direct	++++	0	0	0	±
	P. T.	+++	0	0	0	0
Rtn.	Direct	++++	0	0	0	0
	P. T.	++++	0	0	0	0
Edy.	Direct	++++	0	0	0	0
	P. T.	+++	0	0	0	0
Hbd.	Direct	++++	0	0	0	0
	P. T.	+++	0	0	0	0
Abr.	Direct	++++	±	±	±	±
	P. T.	++	0	0	0	0
Bpo.	Direct	0	0	++++	++++	0
	P. T.	0	0	++	++	0
Jwf.	Direct	0	++++	+	0	0
	P. T.	0	+	+	0	0
Rlg.	Direct	±	++++	++++	++++	+
	P. T.	0	+++	++	++	+
Ash.	Direct	±	+	++	++++	0
	P. T.	0	+	++	++	0
Msl.	Direct	0	±	+	±	0
	P. T.	0	0	+	0	0
Mml.	Direct	±	0	++++	0	0
	P. T.	0	0	+++	0	0
Idh.	Direct	0				
	P. T.	0	0	+	++	0
Lar.	Direct	±	+	+	0	0
	P. T.	0	+	+	0	0
Dkr.	Direct					
	P. T.	0	0	+	0	0
Has.	Direct	0	0	±	++	
	P. T.	0	0	++	+++	
Las.	Direct		++	++	++++	0
	P. T.	0	+	+	0	0
Cap.	Direct					
	P. T.	0	++	+++	++	0

*P.T., Passive transfer.

Human dander extract caused slight skin reactions in many persons not suffering from eczema. Approximately one-half of the patients with an allergic disease other than eczema and one-third of the normal control persons showed a "slight" reaction on direct skin testing. None of the sera from these patients contained skin-sensitizing antibodies. Two of the thirty patients with eczema whose sera sensitized normal skin showed only slight direct skin reactions and these were infants. It is apparent that "slight" reactions may be produced by the human dander extract in some cases and are probably irritative and of no significance, except in the case of infants. This is not unique for human dander, as many other allergens produce reactions in the human skin that are not completely negative, yet are insignificant and unrelated to the allergic condition. The fact that infants and young children react with less intensity to human dander extract by direct skin testing may well be due to the short period of duration of the lesion. It is interesting that the serum of one patient with eczema, here reported, drawn six weeks after the onset of the eczema did not contain skin-sensitizing antibodies, whereas sera drawn seven months later (while the eczema was still present) and fourteen months later (six months after the eczema subsided) did contain skin-sensitizing antibodies. This one observation supports the hypothesis that human dander sensitivity follows the onset of the eczema and is a result rather than a cause of eczema.

Since only three of seventy-five patients with various dermatoses other than eczema showed a significant skin reaction to human dander extract and the sera of two of these three contained skin-sensitizing antibodies, it is apparent that this phenomenon is not based merely upon an inflammatory lesion of the dermis or epidermis.

It may be inferred from these observations that, if the phenomenon is based upon extensive or prolonged inflammation of the skin, it is the patient with an allergic constitutional background who is capable of producing the antibodies to human dander extract, while the nonallergic person is incapable of doing so. Consequently, the persons with eczema, being allergic, produce these antibodies, while those with psoriasis, seborrhea, tinea, and exfoliative dermatitis, being nonallergic, do not. The fact that human dander sensitivity was found in one case of psoriasis and one of tinea, both allergic but without eczema, supports this view.

Since many of the human dander-sensitive patients were nonreactive to other animal epidermals, and many of the patients reactive to lower animal dander were nonreactive to human dander, it may be concluded that general sensitivity to a component, such as keratin, common to all animal danders does not exist.

The observation of Van Leeuwen and his co-workers that almost all allergic and no normal persons react by skin test to human dander extract is not confirmed.

SUMMARY

1. Human dander extract was found to be reactive in certain human skins.

2. Thirty of thirty-three patients with eczema were skin reactive to human dander extract, and their sera contained skin-sensitizing antibodies for it.

3. The results of direct skin tests on various allergic and normal persons are given. Cases of eczema usually showed positive reactions, while the noneczema persons usually showed negative reactions.

4. Only ten of ninety sera from patients with allergic diseases other than eczema contained skin-sensitizing antibodies. None of ten normal nonallergic sera contained skin-sensitizing antibodies.

5. Only three of seventy-five patients with dermatoses other than eczema were significantly reactive to human dander extract.

6. The quantitative studies of the human dander antibody by means of dilution tests are given.

7. Neutralization tests of human dander-sensitive sera, with human dander antigen and house dust antigen are described.

8. No relationship was found between sensitivity to human dander and that to other animal danders.

REFERENCES

1. Van Leeuwen, W. S., Bien, Z., and Varekamp, H.: München. med. Wchnschr. **69**: 1690, 1922.
2. Van Leeuwen, W. S., Bien, Z., and Varekamp, H.: München. med. Wchnschr. **70**: 604, 1923.
3. Van Leeuwen, W. S., Bien, Z., and Varekamp, H.: Klin. Wchnschr. **5**: 1023, 1926.
4. Van Leeuwen, W. S., and Van Niekerk, J.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **63**: 46, 1929.
5. Feinberg, S. M., Taub, S. J., and Unger, L.: Ann. Clin. Med. **5**: 1124, 1927.
6. Murphy, J. A., and Cobe, H. M.: J. ALLERGY **3**: 161, 1931.
7. Keller, P.: Arch. f. Dermat. u. Syph. **148**: 82, 1924.
8. Hampton, S. F., and Stull, A.: J. ALLERGY **11**: 109, 1940.
9. Vickery, H. B., and Leavenworth, C. S.: J. Biol. Chem. **83**: 523, 1929.
10. Block, R. J., and Vickery, H. B.: J. Biol. Chem. **93**: 113, 1931.
11. Block, R. J.: J. Biol. Chem. **104**: 339, 1934.
12. Wilkerson, V. A.: J. Biol. Chem. **107**: 377, 1934.
13. Wilkerson, V. A., and Tulane, V. J.: J. Biol. Chem. **129**: 477, 1939.
14. Pillermer, L., Ecker, E. E., and Wells, J. R.: J. Exper. Med. **69**: 191, 1939.

AN INVESTIGATION OF THE QUESTION OF HISTAMINE TOLERANCE

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THERE is considerable evidence which indicates that liberated histamine is a major factor in the production of anaphylactic symptoms in animals.[1-9] There is suggestive evidence that histamine may also be involved in the genesis of allergic reactions in man.[10-14] On the basis of this latter possibility, interest has been directed toward attempts to treat allergy by procedures which interfere with the action of histamine. One such procedure, which has been reported to be successful clinically, consists of the injection of small, but gradually increasing doses of histamine.[11-24]

Efforts have been made to place these clinical findings on an experimental basis by attempting to demonstrate that a tolerance to histamine may be produced in animals. These experiments have given inconsistent results[25-34] and in many instances bear little resemblance to the "desensitization" procedures which are used clinically. The importance of this latter factor is revealed by a consideration of the three general procedures by which a state of tolerance can be produced in animals. First, single injections of pharmacologic doses of certain drugs produce a fleeting tolerance known as tachyphylaxis. Second, repeated administration of pharmacologic doses of certain drugs, such as morphine, arsenic, or alcohol, produce a more lasting tolerance. Third, single or repeated injections of toxic doses of many drugs produce the "alarm reaction"[35] which is followed by a nonspecific tolerance.

It is obvious that repeated injections of small doses of histamine would be most analogous to the "desensitization" procedure employed clinically. Also, from the standpoint of quantitative evaluation of the results, it is desirable that the response to histamine be a readily measurable one and that the necessity of comparing separate groups of treated and control animals be avoided. Extensive data from experiments fulfilling these three requirements were available to us as a result of repeatedly injecting dogs with histamine for the routine assay of substances inhibiting gastric secretion.[36, 37] The purpose of the present investigation was to determine whether progressive tolerance to histamine occurred in these experiments.

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METHODS

The animals used in these studies were dogs which had been prepared surgically with vagotomized Heidenhain or total pouches of the stomach. The interval between the operation and the beginning of these studies varied in different animals, but in no case was it less than one month. All dogs were trained to stand in stocks for the duration of an experiment.

In all cases, before histamine was administered, the animals were allowed to become basal (secreting no free acid), a condition which is usually maintained for at least four hours if no stimulus is applied. The histamine used was the dihydrochloride; the dosages varied from 0.01 to 0.8 mg. per injection. All injections were made subcutaneously. While the dose of histamine varied from animal to animal, it was kept constant in any given animal from one injection to the next. The dose of histamine was of such a magnitude that the animals were not secreting at their maximal rate.

Following single injections of histamine the gastric juice was collected for a one-hour period. When histamine was given repeatedly at ten-minute intervals, the gastric juice was collected at twenty-minute intervals. The samples were titrated for free or total acidity, using Töpfer's reagent and phenolphthalein respectively. In all cases the responses are expressed as the output of acid in milligrams of HCl.

This investigation may be divided into five groups of experiments. The output of acid in milligrams was determined following the administration of histamine:

1. At ten-minute intervals for a period of four hours. Four total pouch dogs were used, and the secretory response was measured at twenty-minute intervals.

2. At three-hour intervals (A.M. and P.M.). Thirteen total and six Heidenhain pouch dogs were used, and the secretory response was expressed in terms of the miligrams of HCl secreted in one hour.

3. Twice a day for a period of four consecutive days. Eight total and six Heidenhain pouch dogs were used, and the secretory response was expressed in terms of milligrams of HCl secreted in one hour.

4. Twice a day, on alternate days, for a period of three weeks. Five Heidenhain pouch dogs were used, and the secretory response was expressed in terms of the milligrams of HCl secreted in one hour.

5. Twice a day, eight to fifteen times per month, over a period of six months. Five Heidenhain and ten total pouch dogs were used, and the secretory response was expressed in terms of the monthly average of the one-hour secretory responses, in milligrams of HCl.

RESULTS

In the first experiment the twenty-minute secretory response following the subcutaneous injection of histamine at ten-minute intervals was

observed for four hours. It can be seen (Table I) that there is no tendency for the acid secretion to diminish over the four-hour period. If anything, there is a slight rise in response as the experiment progresses.

TABLE I

THE GASTRIC SECRETORY RESPONSE TO HISTAMINE GIVEN EVERY TEN MINUTES (TOTAL POUCH DOGS)

DOG NO.	RATE OF GASTRIC SECRETION (MG. HCl/20 MIN.)											
	20	40	60	80	100	120	140	160	180	200	220	240
T-2	109.0	104.0	102.3	115.8	111.5	117.0	113.5	112.5	119.5	111.0	117.0	116.0
T-3	96.7	88.0	92.5	97.0	98.6	97.0	98.5	103.5	98.2	99.8	106.5	101.5
T-4	114.5	113.5	114.0	113.0	108.0	103.0	106.0	114.0	114.5	111.5	117.5	116.5
T-5	101.5	103.5	113.0	106.5	105.0	103.5	109.0	109.0	110.0	111.0	110.5	107.0
Av.	105.4	102.2	105.4	108.1	105.8	105.1	106.8	109.8	110.6	108.3	112.9	110.3

TABLE II

THE GASTRIC SECRETORY RESPONSE TO HISTAMINE GIVEN AT THREE-HOUR INTERVALS (A.M. AND P.M.)

DOGS	DOG NO.	NO. OF EXPERIMENTS	AV. SECRETORY RESPONSE (MG. HCl/1 HOUR)	
			A.M.	P.M.
Heidenhain pouch dogs	H-1	8	38.2	30.8
	H-2	10	44.1	35.8
	H-3	9	29.4	32.0
	H-4	14	45.2	43.8
	H-5	11	22.9	26.3
	H-6	7	28.2	27.4
	Av.	59	34.7	32.7
Total pouch dogs	T-2	12	81.8	59.8
	T-3	11	72.4	47.5
	T-10	13	58.9	54.4
	T-16	12	131.7	118.9
	T-17	14	172.6	161.2
	T-19	11	44.7	39.8
	T-20	16	112.7	108.1
	T-21	13	107.5	102.3
	T-22	15	106.8	99.5
	T-23	12	153.9	114.3
	T-25	11	154.1	144.3
	T-28	10	141.3	132.5
	T-29	11	160.9	136.2
	Av.	161	115.3	101.4

In the second experiment a comparison was made between the morning and the afternoon responses to the same dose of histamine. Three hours elapsed between histamine injections. It can be seen (Table II) that there is no significant difference between the morning and the afternoon responses in the case of the Heidenhain pouch dogs. However, the afternoon response is significantly less than the morning response in the case of the total pouch dogs.

The third portion of the study deals with the gastric secretory response following the administration of the same dose of histamine twice

a day for four consecutive days. It can be seen (Table III) that there is no particular trend in response in the experiment on the Heidenhain pouch dogs. A tendency toward a diminishing response can be seen in the case of the total pouch dogs.

TABLE III

THE GASTRIC SECRETORY RESPONSE TO HISTAMINE GIVEN TWICE A DAY FOR FOUR CONSECUTIVE DAYS

DOGS	DOG NO.	NO. OF 4-DAY EXPERIMENTS	DAILY SECRETORY RESPONSE (MG. HCl/1 HOUR)			
			1	2	3	4
Heidenhain pouch dogs	H-3	3	41.9	26.3	34.9	38.2
	H-6	2	39.6	40.1	31.2	32.2
	H-4	4	46.2	52.5	44.9	53.0
	H-2	3	60.6	53.5	57.5	31.0
	H-5	2	26.2	45.9	43.9	32.0
	Av.	14	42.9	43.7	42.5	37.3
Total pouch dogs	T-2	6	75.1	64.6	54.1	51.5
	T-3	9	101.7	89.3	96.5	108.7
	T-7	2	54.4	96.5	100.1	53.5
	T-10	7	84.0	82.1	75.5	57.9
	T-17	4	202.6	159.7	165.2	171.1
	T-19	2	48.5	43.4	33.6	42.1
	T-20	2	112.1	105.9	93.1	80.8
	T-22	1	147.0	57.0	56.2	94.0
	Av.	33	103.4	87.3	84.3	82.5

The fourth portion of the study deals with the gastric secretory response following the administration of the same dose of histamine twice a day, every other day, for three weeks. It can be seen (Table IV) that there is no particular trend throughout this experiment.

TABLE IV

THE GASTRIC SECRETORY RESPONSE TO HISTAMINE GIVEN TWICE A DAY EVERY OTHER DAY

(HEIDENHAIN POUCH DOGS)

DOG NO.	DAILY SECRETORY RESPONSE (MG. HCl/1 HOUR)										
	1	3	5	7	9	11	13	15	17	19	21
H-6	33.6	35.4	27.4	31.8	29.5	39.4	47.2	25.2	40.5	36.5	31.0
H-4	54.0	48.8	45.6	48.5	40.5	68.6	43.4	37.2	41.6	45.3	47.5
H-2	63.6	70.3	61.5	41.3	69.5	66.3	51.5	59.5	51.3	50.8	49.0
H-5	23.4	27.4	9.8	16.8	21.2	20.5	36.9	27.4	26.6	28.8	25.5
H-3	29.2	39.4	33.2	21.2	38.3	39.4	33.2	44.9	40.2	9.5	43.7
Av.	40.8	44.3	35.5	31.9	39.8	46.8	42.4	38.8	40.0	34.2	39.3

The purpose of the last portion of the investigation was to determine whether any tolerance to histamine occurs when it is administered over a prolonged period. Since the dogs were started on this experiment at different times of the year, the values are expressed in terms of time from the beginning of the experiment. In the case of the Heidenhain pouch dogs (Table V) there is no tendency toward a decreasing re-

sponse as time progresses. However, in the case of the total pouch dogs (Table VI) there is a definite tendency for the response to decrease after the first month.

TABLE V

AVERAGE MONTHLY SECRETORY RESPONSE TO HISTAMINE GIVEN AT INTERVALS DURING THE MONTH

(HEIDENHAIN POUCH DOGS)

DOG NO.	AV. NO. OF INJECTIONS PER MONTH	AV. MONTHLY GASTRIC SECRETORY RESPONSE (MG. HCl/1 HOUR)					
		1	2	3	4	5	6
H-3	13	38.8	43.5	28.6	40.1	37.1	34.3
H-4	14	43.8	52.7	55.5	57.2	45.6	43.9
H-2	14	31.1	49.9	50.7	58.1	61.6	54.1
H-5	15	22.7	28.7	26.1	35.5	18.9	29.7
H-6	13	26.8	27.5	38.7	30.2	20.9	34.8
Av.	13.8	33.6	41.1	34.1	37.1	33.1	36.6

TABLE VI

AVERAGE MONTHLY SECRETORY RESPONSE TO HISTAMINE GIVEN AT INTERVALS DURING THE MONTH

(TOTAL POUCH DOGS)

DOG NO.	AV. NO. OF INJECTIONS PER MONTH	AV. MONTHLY GASTRIC SECRETORY RESPONSE (MG. HCL/1 HOUR)					
		1	2	3	4	5	6
T-10	11	197.6	163.0	134.2	122.2	137.5	122.2
T-16	12	158.7	104.3	93.2	140.3	126.1	93.4
T-17	11	189.4	173.0	168.8	116.9	110.7	73.5
T-20	9	137.2	113.9	95.2	154.1	149.2	119.5
T-21	10	109.1	78.9	104.1	91.4	61.1	125.3
T-22	11	122.9	99.5	94.7	93.6	50.7	122.6
T-23	8	165.6	123.0	116.1	150.9	100.1	94.4
T-25	10	200.0	141.3	144.3	151.7	162.1	130.1
T-28	10	120.8	83.5	95.8	97.9	89.3	79.7
T-29	10	135.1	111.2	111.1	129.7	121.9	122.7
Av.	10.2	128.0	99.3	96.5	104.1	92.4	90.3

DISCUSSION

A rapidly developing tolerance or tachyphylaxis to the blood pressure effects of histamine has been claimed by certain authors[27-30]; however, other more extensive investigations have failed to confirm these findings.[32, 33] In the present study the evidence indicates that no tachyphylaxis to histamine occurs when it is administered subcutaneously to dogs, as indicated by the gastric secretory response. Even if tachyphylaxis to histamine occurred, there is no reason to believe that such a fleeting tolerance could explain the therapeutic effects which have been claimed for histamine "desensitization" in man.

The question arises as to the significance of the diminishing response in the total pouch dogs over periods of three hours to six months. Since a decreasing response does not occur in the Heidenhain pouch dogs, it

cannot be due to a tolerance to histamine itself. Several factors are operating in the case of the total pouch dogs which offer an explanation for the diminishing response. From a nutritional standpoint, the total pouch dog is a gastrectomized dog. Such dogs have a delicate mineral and nutritional balance.[38] In addition, since the total pouch is much larger than the Heidenhain pouch, more gastric juice is lost. If this loss is excessive, the response to histamine becomes progressively less. The fact that this decrease in response is delayed by the administration of sodium chloride and fluids[39] is evidence that it is due more to the depletion of electrolytes than any tolerance to histamine. Another factor which would tend to produce a diminishing response in the total pouch dogs is the alteration of the acid-base balance, incident to the loss of large amounts of gastric juice. The importance of this factor is brought out by the observation that an artificially produced acidosis or alkalosis gives rise to an apparent tolerance to histamine.[28]

The present observations yield no evidence which would indicate that repeated injections of small doses of histamine induce an elevated histamine tolerance in the dog. Furthermore, tolerance as a result of the repeated administration of pharmacologic doses of histamine has not been observed in man.[40] Hence, the production of an exaggerated tolerance should be excluded as a possible mechanism by which histamine "desensitization" may be conceived to ameliorate certain allergic conditions. It should be emphasized, however, that these experiments were concerned with subjects exhibiting normal tolerances and normal responses to histamine. The possibility remains that repeated histamine injections may restore a histamine hypersensitivity to normal or correct an idiosyncrasy to histamine. If histamine desensitization acts in this way, and only in this way, it is obvious that it should not be equally effective in all allergic patients, since the latter in many instances show perfectly normal responses to histamine. On the other hand, the possibility that histamine "desensitization" interferes with one of the individual stages of the complete allergic or anaphylactic phenomenon[41] cannot be dismissed.

In many of the attempts to modify the histamine tolerance or the anaphylactic reaction in animals by means of pretreatment with histamine, the doses have been far in excess of those used clinically. Doses as great as one-half the minimum lethal dose per injection have been employed over prolonged periods with contradictory results.[31, 34] The positive results which have been reported should probably be attributed to the "alarm reaction,"[35] the essence of which is that a severe, but nonfatal, insult of any type will induce a nonspecific resistance to further insult. This type of nonspecific resistance has been produced with single injections of toxic doses of histamine.[42, 43] It is difficult to believe that this type of resistance has anything in common with clinical "desensitization" induced with minute doses of histamine.

We believe that investigations up to the present time have provided no experimental analogy for the therapeutic effect claimed for histamine "desensitization" in allergy.

SUMMARY

1. The subcutaneous administration of histamine to dogs with total gastric pouches does not result in tachyphylaxis, as measured by the gastric secretory response.

2. A gradual reduction in gastric secretory response is noted in the total pouch dogs when histamine is administered (1) at three-hour intervals, (2) twice a day for a four-day period, and (3) eight to twelve times a month for a six-month period.

3. Under similar conditions, Heidenhain pouch dogs do not show this decreasing response, nor do they show a decreasing response when histamine is given twice daily, on alternate days, for three weeks.

4. It may be concluded that the decreasing response noted in total pouch dogs is related to the greater loss of gastric juice and to the poorer reserve nutrition of the total pouch dogs.

5. The opinion is expressed that animal experiments have so far failed to provide an experimental analogy for the therapeutic effect claimed for histamine "desensitization" in allergy.

REFERENCES

1. Watanabe, K.: Ztschr. f. Immunitätsforsch u. exper. Therap. **69**: 117, 1930.
2. Watanabe, K.: Ztschr. f. Immunitätsforsch u. exper. Therap. **72**: 50, 1931.
3. Dragstedt, C. A., and Gebauer-Fuelnegg, E.: Am. J. Physiol. **102**: 512, 1932.
4. Ibid. **102**: 520, 1932.
5. Bartosch, R., Feldberg, W., and Nagel, E.: Pflüger's Arch. f. d. ges. Physiol. **230**: 129, 1932.
6. Dragstedt, C. A., and Mead, F. B.: J. Immunol. **30**: 319, 1936.
7. Dragstedt, C. A., and Mead, F. B.: J. Pharmacol. & Exper. Therap. **57**: 419, 1936.
8. Ungar, G., and Parrott, J. L.: Ann. de physiol. **13**: 939, 1937.
9. Code, C. F.: Am. J. Physiol. **123**: 40, 1938.
10. Lewis, T., and Grant, R. T.: Heart **11**: 210, 1924.
11. Horton, B. T., MacLean, A. R., and Craig, W. M.: Proc. Staff Meet., Mayo Clin. **14**: 257, 1939.
12. Yates, W. M.: M. Ann. District of Columbia **9**: 165, 1940.
13. Millet, R. F.: Proc. Staff Meet., Mayo Clin. **6**: 93, 1931.
14. Bray, G. W.: J. ALLERGY **3**: 367, 1932.
15. Thiberge, N. F.: J. ALLERGY **6**: 282, 1935.
16. Roth, G. M., and Horton, B. T.: Proc. Staff Meet., Mayo Clin. **12**: 129, 1937.
17. Collens, W. S., Lerner, G., and Fialka, S. M.: Am. J. M. Sc. **188**: 528, 1934.
18. Roth, G. M., and Rynearson, E. H.: Proc. Staff Meet., Mayo Clin. **14**: 353, 1939.
19. Mueller, S.: M. Clin. North America **13**: 1543, 1930.
20. Ernstene, A. C., and Banks, B. J.: J. A. M. A. **100**: 328, 1933.
21. Dzsinich, A.: Klin. Wchnschr. **14**: 1612, 1940.
22. Porch, L. D.: J. Lab. & Clin. Med. **26**: 499, 1940.
23. Farmer, L.: Bull. New York Acad. Med. **16**: 618, 1940.
24. Openchowski, M.: J. M. Soc. New Jersey **34**: 163, 1937.
25. St. Karady, and Bentsath, A.: Ztschr. f. d. ges. exper. med. **100**: 48, 1937.
26. Farmer, L.: J. Immunol. **36**: 37, 1939.

27. Achme, C.: Arch. f. exper. Path. u. Pharmakol. **72**: 76, 1913.
28. Eichler, O., and Killian, H.: Arch. f. exper. Path. u. Pharmakol. **159**: 606, 1931.
29. St. Karady: Arch. f. exper. Path. u. Pharmakol. **180**: 283, 1936.
30. Führer, H.: München. med. Wchnschr. **59**: 852, 1912.
31. Karady, E. S.: J. Immunol. **41**: 1, 1941.
32. Abel, J. J., and Geiling, E. M. K.: J. Pharmacol. & Exper. Therap. **23**: 1, 1924.
33. Guttentag, O. E.: Arch. f. exper. Path. u. Pharmakol. **162**: 727, 1931.
34. Clark, W. G., and MacKay, E. M.: Am. J. Physiol. **126**: 465, 1939.
35. Selye, H.: Endocrinology **21**: 169, 1937.
36. Gray, J. S., Bradley, W. B., and Ivy, A. C.: Am. J. Physiol. **118**: 463, 1937.
37. Gray, J. S., Wieczorowski, E., Culmer, C. U., and Adkison, J. L.: Am. J. Physiol. **129**: 589, 1940.
38. Bussabarger, R. A., Freeman, S., and Ivy, A. C.: Am. J. Physiol. **121**: 127, 1938.
39. Bucher, G. R., Ivy, A. C., and Gray, J. S.: Am. J. Physiol. **132**: 689, 1941.
40. Muether, R. O.: Ann. Int. Med. **13**: 2147, 1940.
41. Dragstedt, C. A.: Ann. Int. Med. **13**: 248, 1939.
42. Selye, H.: Klin. Wchnschr. **17**: 666, 1938.
43. Selye, H.: Canad. M. A. J. **34**: 706, 1936.

INVESTIGATIONS ON THE PRESENCE OF FORSSMAN ANTIGEN IN RAGWEED POLLEN

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THE problem of the presence or absence of Forssman antigen in ragweed pollen has been the subject of several investigations. Garvin and Kurland[1] immunized rabbits with ragweed pollen without obtaining sheep cell hemolysins of the Forssman type. Sinclair and Thomas[2] observed an increase in titer of heterophile antibodies in serum of allergic patients in comparison with nonallergic patients. However, those observations were irregular. Recently Sammis[3] came to the conclusion that Forssman antigen is present in ragweed pollen. Sammis' statements are based, according to her opinion, upon immunization experiments in rabbits that produce heterophile antibodies when treated intravenously with ragweed pollen. Human subjects, however, in spite of prolonged treatment, did not show any increase in heterophile antibodies. Eagle, Arbesman, and Winkenwerder[4] showed that rabbits treated intravenously with ragweed pollen produced a fairly high titer of antibodies as shown by precipitation and complement fixation tests. Such rabbit sera were capable of giving a positive Prausnitz-Kuestner reaction in man. Because of the difference in opinion as to the presence or absence of Forssman antigen in ragweed pollen, the investigations herewith reported were undertaken.

METHODS AND MATERIALS

1. Preparation of Extract.—A 10 per cent extract of short ragweed pollen was prepared in the following way. A 10 per cent suspension (weight to volume) of defatted short ragweed pollen in distilled water was shaken, covered with a layer of toluol, and allowed to stand in the refrigerator for twenty-four hours. This mixture was then filtered through paper and finally through a Seitz filter. Cultures, both aerobically and anaerobically, proved to be sterile. This extract was then lyophilized in 10 c.c. amounts in each ampoule and the powdered extract sealed. As the extract was needed, sterile physiologic saline was added to the ampoule in the 10 c.c. amount and was used immediately for injection of rabbits.

Total nitrogen was 1.78 mg. per cubic centimeter, and protein nitrogen, as precipitated by phosphotungstic acid, 0.85 mg. per cubic centimeter.

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2. Method of Immunization.—Three rabbits were injected intravenously and subcutaneously three times weekly with progressively increasing amounts of the 10 per cent defatted lyophilized short ragweed pollen extract. The period of immunization was thirty-eight days. A trial bleeding of each rabbit was done at this time and the antibody titers determined. After a rest period of four weeks, the rabbits were reinjected intravenously three times weekly for eight doses. The typical schedule is given by way of illustration in Table I.

TABLE I

METHOD OF IMMUNIZATION

DAY NO.	ANTIGEN (10% EXTRACT SHORT RAGWEED POLLEN) (C.C.)
1	0.25
3	0.5
5	0.75
8	1.0
10	1.25
12	1.5
15	1.8
17	2.0
19	2.25
22	2.5
24	2.5
26	2.5
29	2.5
31	2.5
33	2.5
35	2.5
37	Bleeding
Rest Period 28 Days	
63	1.0*
65	1.5
67	2.0
70	2.25
72	2.5
74	3.0
77	3.0
79	4.0
Final bleeding	

*Intracutaneous injection.

EXPERIMENTAL DATA

1. Examination of Ragweed Antisera for Precipitins.—In order to determine the titer of precipitins formed during the process of immunization, the following test was set up. Decreasing amounts of ragweed antiserum (diluted with physiologic saline), volume 0.4 c.c., were mixed with 0.1 c.c. of the 10 per cent short ragweed extract. The tubes were placed in the icebox overnight. The resulting precipitation recorded is given in Table II.

Table II shows that all three ragweed antisera gave a definite precipitation in dilutions of 1:16, and two of them gave some precipitation in dilutions of 1:32 with ragweed extract.

2. Examination of Ragweed Antisera for Sheep Cell Hemolysins.—The presence of sheep cell hemolysins in the three antiragweed rabbit

TABLE II

PRECIPITATION OF RAGWEED POLLEN EXTRACT BY RAGWEED ANTISERUM

NO.	DILUTIONS OF SERUM	0.1 C.C. OF 10% SHORT RAGWEED EXTRACT ADDED TO 0.4 C.C. OF RABBIT SERUM		
		481	482	483
1	1:2	++++	++++	++++
2	1:4	++++	++++	++++
3	1:8	+++	+++	+++
4	1:16	++	++	+++
5	1:32	+	–	±
6	1:64	–	–	–
7	0	–	–	–

Demonstration of precipitating antibodies to short ragweed pollen in rabbit antisera produced by the intravenous injection of short ragweed pollen extract.

++++, Very strong precipitation; +++, strong precipitation; ++, moderate precipitation; +, slight precipitation; ±, faint precipitation; –, no precipitation.

All three ragweed antisera gave a definite precipitation in dilutions of 1:16.

sera was determined. Decreasing amounts of rabbit serum, volume 0.2 c.c., were mixed with 0.2 c.c. of a 1:20 dilution of guinea pig serum and 0.1 c.c. of a 5 per cent washed sheep cell suspension. As a positive control a 1:100 dilution of the serum of rabbits immunized with sheep cells was used. The reading given in Table III was obtained after thirty minutes' incubation at 37° C.

TABLE III

LYSIS OF SHEEP CELLS BY RABBIT SERUM IN THE PRESENCE OF COMPLEMENT

NO.	DILUTION OF SERUM	RABBIT SERUM			SHEEP CELL ANTISERUM DILUTED 1:100
		481	482	483	
1	undiluted	c	ac	c	c
2	1:2	c	m	c	c
3	1:4	ac	m	ac	c
4	1:8	m	tr	ac	c
5	1:16	ac	0	tr	c
6	1:32	tr	0	0	c
7	1:64	0	0	0	m
8	1:128	0	0	0	m
9	0	0	0	0	0

The tables show that hemolysis obtained by the three ragweed antisera is definite only in higher concentrations of the serum.

c, Complete hemolysis; ac, almost complete hemolysis; m, moderate hemolysis; tr, trace of hemolysis; 0, no hemolysis.

As can be seen from Table III, hemolysis produced by the three ragweed antisera is definite only in higher concentrations of the serum. However, the hemolysin titer is similar to that of rabbits immunized with any kind of foreign protein and cannot be compared at all with that of a typical Forssman antiserum. Sammis,[3] too, observed that the heterophile titer of her ragweed antisera was low. In order to prove that the Forssman antibodies present in rageed antisera were caused by Forssman antigen present in ragweed pollen, the so-called hemolysis inhibition test was employed. This test is based upon the following facts. Forssman antibodies obtained by immunization are neutralized

by the addition of their homologous antigen. Consequently, sheep cells, when added after neutralization has occurred, are not dissolved any more in the presence of complement. The neutralizing potency of such an antigen is very marked and can easily be demonstrated. If the sheep cell hemolysins present in ragweed antisera should be produced because of the presence of some trace of Forssman antigen in ragweed pollen, the addition of the ragweed pollen should be capable of neutralizing heterophile antibodies present in ragweed antiserum. In order to obtain information on that question, the following experiment was set up.

Decreasing amounts of ragweed extract, volume 0.2 c.c., were mixed with 0.2 c.c., of a 1:2 diluted antiragweed rabbit serum (inactivated for twenty minutes at 56° C.). This dilution was found to represent about two hemolytic doses. The mixtures were kept for thirty minutes in the incubator at 37° C. After this time, to each tube was added 0.2 c.c. of a 1:10 dilution of guinea pig serum and 0.2 c.c. of a 5 per cent sheep cell suspension. The reading was obtained after complete hemolysis had occurred in the control tube which took about thirty minutes.

TABLE IV

INHIBITION OF LYSIS OF SHEEP CELLS BY RAGWEED ANTISERUM DILUTION (1:2) AND COMPLEMENT IN THE PRESENCE OF RAGWEED EXTRACT

NO.	DILUTION OF 10% SHORT RAGWEED EXTRACT	DEGREE OF HEMOLYSIS*
1	Undiluted	0
2	1:4	0
3	1:16	0
4	1:64	0
5	1:256	ac
6	1:1,024	ac
7	1:4,000	c
8	0	c

*Readings obtained after one-half (½) hour when complete hemolysis had occurred in the control tube No. 8.

Ragweed antigen in a dilution of 1:64 completely inhibits lysis of sheep cells present in ragweed antiserum No. 481.

As can be seen from Table IV ragweed antigen in dilutions up to 1:64 completely inhibits lysis of sheep cells present in ragweed antiserum No. 481, while partial inhibition of hemolysis is to be observed in dilutions up to 1:1,024. The hemolytic activity of normal rabbit serum, when examined under identical conditions, was not inhibited to the same extent as that of the ragweed antiserum, thus confirming Sammis' observations.

3. Examination of Ragweed Antisera for Complement-Fixing Antibodies.—One might be inclined to draw the conclusions from these findings that neutralization of the sheep cell hemolysins present in ragweed antiserum was caused by the Forssman antigen content of ragweed pollen. However, there is a second possibility to be considered. The lack of hemolysis as demonstrated in the preceding experiment might

be caused by a deviation of complement due to an antigen-antibody reaction rather than by direct neutralization of sheep cell hemolysins. From previous experiences[4] it is known that ragweed antiserum contains complement-fixing antibodies. The next experiment to be reported deals with the problem whether or not complement fixation might occur under similar experimental conditions. The experiment shown in Table V differs from the preceding one in only one respect, sheep cells sensitized with their homologous amboceptor were used instead of sheep cells suspended in saline solution.

Three-tenths cubic centimeter of undiluted inactivated antiragweed serum No. 481 was placed in each of five tubes. To the first four tubes was added 0.3 c.c. of varying dilutions of fresh 10 per cent short ragweed extract. To the fifth one saline solution was added. The mixtures were shaken and placed in the incubator for thirty minutes at 37° C. To decreasing dilutions of these mixtures (volume 0.2 c.c.) were added 0.2 c.c. of a 1:10 dilution of complement and 0.2 c.c. of 5 per cent sheep cells sensitized with amboceptor diluted 1:3,000, representing two amboceptor units. The experiment was kept at 37° C. Two readings were recorded (Table V); the first was taken after fourteen minutes, and the second, after thirty minutes.

TABLE V

COMPLEMENT FIXATION BY RAGWEED ANTISERUM AND RAGWEED EXTRACT

NO.	DILUTION OF MIXTURES	SERUM WITH EXTRACT										SALINE	
		1:4		1:16		1:64		1:256		1:1,024			
1	Undiluted	0	0	0	0	0	m	ac	c	c	c	c	c
2	1:2	0	0	0	0	tr	c	c	c	c	c	c	c
3	1:4	0	tr	0	m	m	c	c	c	c	c	c	c
4	1:8	tr	m	tr	ac	ac	c	c	c	c	c	c	c
5	1:16	m	c	m	c	ac	c	c	c	ac	c	ac	c
6	1:32	ac	c	m	c	ac	c	ac	c	m	c	m	c
7	1:64	m	c	m	c	m	c	ac	c	m	c	m	c
8	0	m	c	m	c	m	c	m	c				

The first and second readings were taken fourteen and thirty minutes, respectively, after complement and sensitized sheep cells were added.

Complement fixation occurs under conditions similar to those used in the hemolysis inhibition test. A strong reaction is obtained in dilution of the extract up to 1:64.

The experiment proves that complement fixation occurs under conditions similar to those used in the hemolysis inhibition test. A strong reaction is obtained in dilution of the extract up to 1:64. On quantitative examination the mixture consisting of an extract dilution of 1:4 and serum gives a stronger reaction than a mixture consisting of serum and a 1:64 dilution of the extract. A conclusion to be drawn from the experiment is that a deviation of complement might occur under the experimental conditions chosen. In other words, the inhibition of hemolysis as shown in Table IV might be explained by the occurrence of an antigen-antibody reaction destroying the complement necessary to dissolve sheep cells in the presence of heterophile antibodies. Of course,

the order of experiment given in Table V does not constitute ideal conditions for a complement fixation test. Much stronger reactions might be obtained if the customary type of experiment is employed as shown in the following experiment.

Decreasing amounts of ragweed extract, volume 0.2 c.c., were mixed with 0.2 c.c. of ragweed antiserum No. 481 in a dilution of (a) 1:2 and (b) 1:18. Then 0.2 c.c. of a 1:20 diluted guinea pig serum was added. The mixtures were kept in the icebox for one hour and placed in the incubator for forty-five minutes. Then 0.4 c.c. of sensitized sheep cells were added. Table VI represents the reading obtained after twenty-five minutes' incubation at 37° C. after which the controls had cleared up completely.

TABLE VI

TITRATION OF RAGWEED ANTISERUM AGAINST RAGWEED EXTRACT BY MEANS OF COMPLEMENT FIXATION

NO.	DILUTION OF RABBIT SERUM 481	DILUTIONS OF EXTRACT										SALINE
		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	
1	1:2	0	0	0	0	0	0	tr	m	ac	c	c
2	1:18	0	0	0	0	0	0	0	0	m	c	c

As can be seen from the experiment, a strong complement fixation test might be obtained when ragweed antiserum and ragweed extract are mixed under suitable experimental conditions. An extract dilution up to 1:2,560 might definitely show some reaction when a 1:18 dilution of the serum is used, while a stronger concentration indicates the interference of some kind of surplus inhibition. Consequently, the ragweed antigen constitutes a suitable antigen in complement fixation tests with ragweed immune serum.

DISCUSSION

The presence of Forssman antigen in cells and tissues can be demonstrated by immunization of rabbits. The appearance of sheep cell hemolysins in the serum of the rabbit following treatment is considered to be an indicator for the presence of Forssman antigen in the material under investigation. In most of the cases, a strong antibody response is obtained because of the well-known antigenic potency of the Forssman antigen. In instances where only small traces of the Forssman antigen are available, as in the case of the blood group substance A, a high titer of Forssman antibodies is produced upon immunization of rabbits. On the other hand, it is an old experience that treatment of rabbits with all kinds of antigens lacking Forssman antigen might result in a three- to fivefold increase of the original hemolysin titer. This increase is generally ascribed to be a nonspecific side effect of all im-

munization procedures. However, the possibility cannot be denied that occasionally a slight increase in titer might be due to the specific effect of immunization caused by a trace of Forssman antigen present in the material used for immunization purposes. In those instances it might be difficult to prove that an insignificant increase in sheep cell hemolysins constitutes a specific effect of immunization. Absorption experiments might overcome some of the objections that may be raised against the specific nature of such an increase. In this respect it seems to be rather difficult to use the "inhibition of hemolysis" test as an instrument to prove absorption of Forssman antibodies if complement fixation occurs simultaneously. Lack of hemolysis of sheep cells in the case of the inhibition test consequently might be caused by the destruction of complement following an antigen-antibody reaction between the ragweed antigen and the ragweed antibody present in ragweed immune serum. Observations on ragweed pollen failed to indicate any presence of Forssman antigen. We, therefore, feel that there is no sufficient experimental proof as yet available to assume that ragweed pollen contains Forssman antigen.

SUMMARY

1. Rabbits immunized with ragweed pollen extract produce ragweed antibodies.

2. Ragweed antibodies are easily demonstrable by precipitation and complement fixation tests.

3. The increase in titer of Forssman antibodies in such rabbit sera is not significant.

There is no evidence for the assumption that Forssman antigen is present in ragweed pollen.

REFERENCES

1. Garvin, P. D., and Kurland, S. K.: J. ALLERGY **7:** 475, 1936.
2. Sinclair, M. W., and Thomas, J. W.: J. ALLERGY **10:** 228, 1939.
3. Sammis, F. E.: J. Exper. Med. **71:** 591, 1940.
4. Eagle, H., Arbesman, C. E., and Winkenwerder, W. L.: J. Immunol. **36:** 425, 1939.

ALLERGIC ASTHMA AND RHINITIS DUE TO KAMALA POWDER

McKinley London, M.D., Cleveland, Ohio

THE *Mallotus philippinensis* tree, native to Abyssinia, the East India Islands, China, and Australia, is 15 to 20 feet in height and yields capsules the size of small cherries. The capsule is covered by a finely granular reddish brown powder known as kamala powder. This material is separated by the agitation of the capsules over fine wicker work.

Kamala powder has been used for many centuries in Asia as a vegetable dye. It formerly was generally employed as a vermifuge and teniacide in humans but has been replaced by more effective remedies. It is still used in India for tapeworm internally and applied externally in cases of scabies and ringworm. In this country it is used extensively as an anthelmintic in poultry husbandry. It is administered several times a year to poultry, directly in tablet and capsule form, or indirectly by mixing it with their feed.

Kamala powder is antigenic as illustrated by the following two cases.

CASE REPORTS

Case 1.—M. T. complained of rhinorrhea and asthmatic attacks. Other than these he had no allergic manifestation. His family history shows a brother who is similarly afflicted. His past history showed the usual childhood diseases. His tonsils had been removed one year before he was seen. A polyp was removed from the left nostril two months before admission. For the past six or seven years he has been suffering from a rhinitis which is perennial and worse in winter. He is certain that contact with acacia powder will cause rhinitis and asthma. Fresh cherries and cherry wine will cause nausea, vomiting, and asthma. He is able to eat cooked cherries without ill effects. He has been employed for the past thirteen years in making medicinal tablets for a pharmaceutical company. While making pills, one constituent of which was kamala powder, he was seized with an attack of rhinitis and asthma. This happened often except on the occasions when he wore a mask which filtered the inspired air.

Physical examination showed the patient to be a white male, aged 31 years, weighing 168 pounds. The area over the left antrum revealed diminished transillumination. The heart and lungs contained no abnormal findings; blood pressure was 124/76. The Wassermann reaction was negative, and the urine showed nothing abnormal. Skin tests, both scratch and intradermal, showed many positive reactions. The only ones considered of immediate significance were cherry, acacia, and kamala powder. One-tenth cubic centimeter of his blood serum was transferred to the skin of a normal person. The next day a scratch test with moistened kamala powder was made at the site of transfer. A prompt reaction of wheal and erythema developed. The patient has ceased making tablets and is now free from symptoms.

From the Allergy Clinic, St. Luke's Hospital, Cleveland.

CASE 2.—J. T., a brother of M. T., complained of rhinorrhea and asthma. Until the present complaint, he had been in perfect health except for influenza in 1928. His tonsils were removed in 1933. He was employed in the same capacity as his brother, namely, the manufacture of pharmaceutical tablets. Almost immediately after exposure to kamala powder his eyes itched; he beğan to sneeze; he had rhinorrhea; and his nose became blocked. Later he noted dyspnea which lasted for twelve hours. Coming into a room where kamala was used would cause symptoms. This happened on many occasions, and his symptoms seemed so directly associated with kamala powder contact that he was convinced of its etiologic relationship. He had had no other allergic manifestations.

Physical examination revealed a white male, aged 29 years, weighing 198 pounds and 5 feet 11 inches in height. The nose showed no abnormalities. The frontal sinuses and both antra transilluminated well. The chest was well formed and symmetrical; there were equal expansion, no impairment of percussion note, normal vesicular breath sounds, and no adventitious sounds. The heart rate was 66; there were no enlargement, no murmurs, and sounds of good quality. The blood pressure was 120/88. The urine and Wassermann tests were negative. Scratch skin tests showed many one- to two-plus reactions, none of which were of any clinical significance. However, his reaction to a scratch test with moistened kamala powder was an irregular wheal, measuring 1.5 by 1 inch, surrounded by a large zone of erythema. The Prausnitz-Küstner passive transfer reaction was positive. A clinical trial with kamala was done by insufflation of the crude powder into one nostril and into the trachea. The nasal mucous membrane began to discharge a clear watery fluid, became pale, and swelled with such rapidity that in a short time the entire cavity was blocked. Soon after insufflation of the trachea, he began to cough. In about twenty minutes he noted a pronounced difficulty in breathing, accompanied by dry râles throughout the chest. He was readily relieved by 5 minims of epinephrine. He was given ephedrine to use if required and was allowed to return home. He used the ephedrine from time to time and claimed that his chest was never entirely free from obstruction for thirty-two hours. He has refrained from contact with kamala and has had no further trouble.

SUMMARY

Kamala powder, which is used extensively as an anthelmintic in poultry husbandry, is described. Two cases are presented illustrating its antigenic properties. Its antigenicity is proved by (1) contact, in one case premeditated, causing typical allergic symptoms of rhinorrhea and asthma in two predisposed persons, (2) cessation of symptoms on elimination of the suspected substance, and (3) the presence of transferable rēagins.

Selected Abstracts

Asthma and Hay Fever

UNDER THE DIRECTION OF SAMUEL M. FEINBERG, M.D., AND
THEODORE B. BERNSTEIN, M.D., CHICAGO

Studies on Blood Histamine in Patients With Allergy. II. Alterations in the Blood Histamine in Patients With Allergic Disease. Rose, B.: J. Clin. Investigation **20**: 419, 1941.

A group of eighty patients suffering with allergic diseases including asthma, urticaria, angioneurotic edema, eczema, vasomotor rhinitis, and colitis was studied with regard to the histamine content of the blood. In the asthma group, it was found that some of the patients had a blood histamine level below normal, while others had a level greater than normal. Most of the patients showed a histamine content within the normal range. Patients less than 16 years of age tended to have a high histamine level. In all of these patients with the exception of those with urticaria there was a marked fluctuation of the histamine values, as compared with the stability of the blood histamine level in normal persons. These fluctuations in the asthma, eczema, and vasomotor rhinitis cases are not necessarily correlated with the symptoms. The histamine level in the angioneurotic edema cases generally dropped with the onset of symptoms and returned to normal when the symptoms subsided. The results do not warrant the conclusion that histamine is released during the attack in the allergic diseases studied by the author.

Studies With Antigens. IX. Treatment With Purified Extracts of House Dust. Efron, B. G., Boatner, C. H., and Everett, P.: New Orleans M. & S. J. **93**: 516, 1941.

Seventy-two patients, who were sensitive to house dust and who had asthma or hay fever, or both, were treated with injections of purified house dust extract. Treatment was carried on for nine to eighteen months, and the injections were given at intervals of every other day at first and later once a week with the more concentrated material. No attempt was made to instruct the patients in the methods of dust precaution. Definite relief from symptoms was obtained in 84 per cent of the cases; 51 per cent had marked relief and 33 per cent reported moderate improvement.

Use of an Emulsified Extract in the Treatment of Ragweed Pollinosis. A Preliminary Report. London, M., and Davy, E. R.: Ohio State M. J. **37**: 446, 1941.

London and Davy treated a group of twenty ragweed hay fever patients in 1940 with a ragweed extract emulsion consisting of 4 per cent ragweed extract emulsified with olive oil and 1 per cent oleic acid. The treatment in most cases consisted of ten injections given intramuscularly at weekly intervals, with a dose schedule in units as follows: 100, 500, 1,500, 5,000, 10,000, 15,000, 20,000, 30,000,

45,000, and 60,000 units. The volume of each injected dose was 1 c.c. or more. The authors reported that good clinical results were obtained in this group as compared with patients treated with aqueous extracts. They claimed that only two mild constitutional reactions occurred during the course of treatment and epinephrine was not given. Local reactions were common, for those patients who received 40,000 to 60,000 units complained of swelling of the entire arm to the wrist precluding bending at the elbows. Another patient, seen three days after the injection, had an arm twice the circumference of the untreated arm. Two patients absented themselves from treatment because their arms were still swollen. In several cases, a considerable swelling from the previous injection made it necessary to omit the next weekly injection. In two instances, a sterile inflammatory reaction the size of a small tangerine developed, and, on incision, unabsorbed oil escaped.

Complement Fixation With Specific Allergens in Hay-Fever. I. Problem, Procedure, Diagnostic Value. Hensel, M. E., and Sheldon, J. M.: J. Lab. & Clin. Med. **26**: 1586, 1941.

The authors used the complement fixation method of Albus to determine the amount of specific antibodies in the blood of twenty-five hay fever patients. These were subjects who were sensitive to ragweed or grass pollen, or both, and who had not had any desensitization therapy for a long time prior to the experimental work. The sera of twenty-five normal persons were also tested. Complement fixation occurred with higher dilutions of the pollen antigens in the hay fever group, as compared with the control subjects, who gave only an occasional complement fixation reaction with highly concentrated extracts.

A New Treatment for Hay-Fever. Elsbach, E. J.: New York State J. M. **41**: 1248, 1941.

The author treated seventy-five patients suffering from both seasonal and perennial rhinitis with injections of a filtrate of *Bacillus coli*. Injections were given intramuscularly at weekly intervals, and usually between eight to twelve injections sufficed to produce results. Fifty-three, or 70 per cent, of the seventy-five patients were "cured"; twenty-one cases, or 28 per cent, were improved, and one patient had no result. Good results were also obtained in cases of allergic asthma and allergic eczema. The author states that among the advantages of this "new biologic treatment" is included the fact that "tests to find out which allergen is at fault are not necessary."

A Proposed Simple Method of Determining Clinical Hypersensitiveness in Allergic Patients. Wittich, F. W., and Boyle, S. M.: Journal-Lancet **61**: 285, 1941.

The authors observed the effect on the capillaries of the foot web of the frog of high dilutions of histamine and of plasma prepared as follows from ragweed hay fever patients. Citrated blood from an untreated hay fever patient was incubated with an aqueous ragweed extract and the plasma separated by centrifugation. A drop of this plasma placed upon the foot web caused dilatation of the capillaries and increased circulation, which lasted for fifteen to twenty minutes. A similar result was obtained by the use of a nonallergic patient's plasma, but the effect was definitely weaker. In six cases studied so far, five ragweed hay fever patients and a child with severe eczema due to wheat, there was a definitely greater response as compared with the control experiment.

Preparation of a Slowly Absorbed Pollen Antigen. A Preliminary Report. Rockwell, G. E.: Ohio State M. J. **37**: 651, 1941.

A relatively insoluble salt of ragweed pollen extract was prepared by precipitating the extract with hydrochloric acid. The precipitate was finely suspended in sterile saline solution and dilutions were made for treatment of a series of hay fever cases. Good clinical results were obtained. Since the precipitate is relatively insoluble, Rockwell claims it is slowly absorbed and less likely to give systemic reactions; consequently, the dosage can be more rapidly increased.

Dermatology

UNDER THE DIRECTION OF MARION SULZBERGER, M.D., NEW YORK, AND JOSEPH GOODMAN, M.D., BOSTON

Bacterial Allergy: An Etiologic Factor in Dermatitis Herpetiformis. Callaway, J. L., and Sternberg, T. H.: Arch. Dermat. & Syph. **43**: 956, 1941.

The authors report a case of dermatitis herpetiformis which was treated unsuccessfully for six years with the usual remedies for this disease. It was noted, however, that the patient had a low-grade bronchiectasis. A pure culture of pneumococcus Type VII was obtained from material recovered through a bronchoscope. Both patch and intradermal tests with 0.1 c.c. of a vaccine made from a culture of this organism (1 million organisms to the cubic centimeter) resulted in erythema and edema which were 7 cm. in diameter and presented bullae in the central portions. Similar tests on control patients gave negative results. Desensitization with the vaccine resulted in marked improvement which was not complete, however, until the vaccine was given intravenously. Discontinuation of the treatment resulted several times in mild recurrences.

Contact Dermatitis Due to Underwear. Neilson, A. W., and Reiches, A. J.: Arch. Dermat. & Syph. **44**: 218, 1941.

Fourteen patients with dermatitis caused by the wearing of new unwashed shorts were tested with the various materials used as finishes on the shorts. These materials were tested because it was found that the patients presented positive patch tests to the unwashed shorts, negative patch tests to the washed material. In each instance it appeared that the commercial oleoresin used in the finish of the cloth was the cause of this dermatitis. Abietic acid was not found to be of importance in this group of patients or in the control groups.

The Fate of Treponema Pallidum Inoculated Subcutaneously Into Immune Rabbits. Reynolds, F. W.: Bull. Johns Hopkins Hosp. **69**: 53, 1941.

A group of rabbits which had been inoculated intratesticularly with the Nichols strain of *Treponema pallidum* were treated with arsphenamine approximately six months after the infection had been established. Reinoculation of these animals subcutaneously (by implantation) with the same strain was not followed by a local lesion. Active organisms could be demonstrated in the implant for four days but could be demonstrated by transfer to normal animals for only two days. In no instance could organisms be demonstrated by transfer in the regional lymph nodes of the immune animals.

In a control group of normal animals there was likewise no chancre at the site of the implant. However, the implants presented active organisms by dark-field examination for fourteen days (as long as studied), and in each instance there were active organisms in the regional lymph nodes.

It seems probable that in the reinoculated animals under the conditions of this experiment the organisms are localized at the site of inoculation and subsequently destroyed by the immune mechanisms of the host.

Studies in Adhesive Tape Irritation. Sheldon, J. M., Hensel, H. M., and Blumenthal, F.: J. Invest. Dermat. 4: 295, 1941.

The authors studied adhesive tape eruption in a large series of persons. Sex seemed to be of relatively little importance in regard to adhesive eruption. In children there was a larger percentage of reactions than in adults, although in children the reactions tended to be relatively mild. A group of allergic persons (most of whom were atopic) presented reactions to adhesive tape in about the same proportion as a group of normal controls.

Microscopic studies of the reactions to adhesive tape threw little light on the problem of the importance of mechanical as compared to allergic reactions.

One hundred and twenty-six persons who showed reactions to adhesive tape and sixty-eight who showed no reactions to adhesive tape were tested with nineteen ingredients which are commonly used in making such tape. There were 236 reactions in the sensitive group as compared to forty-four in the nonsensitive group. Pararubber produced the largest number of reactions in each group (fifty-seven and nineteen, respectively). Other rubbers were likewise frequently responsible for the production of reactions.

The next most frequent reaction was that to Burgundy pitch, followed by various rosins and by olibanum. On the basis of these findings a new type of tape was prepared which gave fewer patch test reactions when compared with commercial tape on the market. Details of the composition of this new tape are not given.

Observations on the Oral Administration of Ragweed Oil in Patients Hypersensitive to Ragweed Oil. Sheldon, J. M., and Blumenthal, F.: Am. J. M. Sc. 202: 98, 1941.

The authors treated sixteen patients with contact dermatitis caused by ragweed pollen. All patients gave a strongly positive patch test reaction to oil of ragweed in a dilution of 1:1,000 or higher. Treatment was started with the oral administration of a drop of that dilution which gave a faintly positive patch test reaction. It was demonstrated that in many instances the sensitivity to ragweed pollen was appreciably reduced, as evidenced by patch tests with graded dilutions performed before and after the administration of the extract by mouth. In several instances, however, the patch test sensitivity failed to show a quantitative reduction in spite of the fact that the patients remained symptom free after treatment. Of the sixteen patients treated, twelve experienced satisfactory results. Unfortunately, the duration of treatment is not indicated in the report.

Studies With Paraphenylenediamine in Fur Workers. Silverberg, M. G., and Heimann, H.: J. Invest. Dermat. 4: 193, 1941.

Ten patients with asthma who in their daily work came in contact with furs dyed with paraphenylenediamine were tested by patch and scratch tests with paraphenylenediamine in various forms. In six of the ten patients the asthma

was definitely related to employment. In the group of asthmatic patients all scratch tests to paraphenylenediamine were negative. On the other hand, patch tests of these ten individuals resulted in strongly positive reactions to paraphenylenediamine in four instances. In one of these four persons there was a history of recurrent dermatitis, probably caused by paraphenylenediamine. In the other three there was no history of dermatitis.

Fifty workers in a fur dying establishment, none of whom ever presented dermatitis or asthma, were patch-tested with paraphenylenediamine and related substances. Only three showed weak reactions. No explanation is at hand for the finding that a relatively large percentage of persons with asthma presumably due to paraphenylenediamine give positive reactions to patch tests but negative reactions to scratch tests with this substance.

In this study the paraphenylenediamine for scratch testing was used in the following forms: (1) dissolved in various concentrations in human serum, (2) dissolved in water and boiled for 0.5 hour, (3) oxidized by adding hydrogen peroxide to the water solution, and (4) alkalinized by adding sodium hydroxide to the serum solution.

Immunology

UNDER THE DIRECTION OF MATTHEW WALZER, M.D., AND MAX HARTEN, M.D., BROOKLYN

Effect of Trypsin Upon Blood Histamine of Rabbits. Dragstedt, C. A., and Rocha e Silva, M.: Proc. Soc. Exper. Biol. & Med. **47**: 420, 1941.

The intravenous injection of from 3 to 7 mg. per kilogram of crystalline trypsin into rabbits is followed by a prompt reduction in the total blood histamine with an associated leucopenia. The addition of trypsin to heparinized rabbit blood in vitro produced a marked shift of the histamine from the cells to the plasma.

The effects of trypsin upon the blood histamine of rabbits were similar to those produced by the addition of specific antigen to the blood of sensitized rabbits.

The Presence and Distribution of Histamine in Blood. Minard, D.: Am. J. Physiol. **132**: 327, 1941.

A modified method for the extraction of blood histamine by electrodialysis and assay on isolated guinea pig intestinal strips is described.

Studies on the distribution of histamine in rabbit blood indicate that the histamine present is localized to a large extent (97 to 98 per cent) in the platelets. On the basis of platelet volume and platelet histamine determinations, rabbit platelets were found to contain approximately 400 gamma of histamine per milliliter. Experiments on dogs indicate that, in this species, the platelets contribute relatively little to the total blood histamine.

The author suggests that conclusions based on blood histamine studies in a particular species cannot be applied to other species.

The Effects of Ether Anaesthesia on Anaphylaxis. Koontz, A. R., and Shackelford, R. T.: Anesth. & Analg. **19**: 194, 1940.

The literature on the effects of anesthesia on anaphylaxis is reviewed.

The best technique for the study of the effects of anesthesia on anaphylaxis necessitated the use of guinea pigs weighing not less than 650 Gm. Three weeks

after sensitization by the subcutaneous route the animals were shocked by an intrajugular injection of 2 ml. of horse serum. Seven of the fifty-seven animals (12.2 per cent) tested while under ether anesthesia died in anaphylactic shock. Thirty-two of the sixty-six animals (48.5 per cent) tested without anesthesia died in anaphylactic shock.

The authors conclude that either anesthesia gives positive protection against anaphylaxis in guinea pigs. No convincing evidence that this finding applied to human beings has been reported. The common practice of dispensing with serum sensitivity tests prior to the administration of antitoxin to patients under anesthesia is not justified.

Effect of Histaminase on the Shwartzman Phenomenon. Bosse, M. D.: J. Lab. & Clin. Med. **26**: 1432, 1941.

Little or no effect on the production of the Shwartzman phenomenon in rabbits was observed following pretreatment of such animals with large doses of histaminase. The histaminase was administered orally, subcutaneously, or intravenously just prior to, several hours previous to, or immediately following either the preparatory or provocative injection.

Precipitin Formation in the Guinea Pig. Colwell, C. A., and Youmans, G. P.: J. Infect. Dis. **68**: 226, 1941.

Circulating precipitin could not be detected in ten guinea pigs given multiple intraperitoneal injections of plain or alum-precipitated crystalline egg albumen. The possible influence of the route of injection is now being investigated since neither the amount of antigen injected nor the interval between injections appears to be a factor in the failure of this animal to produce detectable precipitin.

Twenty guinea pigs given multiple intraperitoneal injections of sheep serum produced strongly flocculating antisera. Antiserum dilution titers were as high as 1:3,000 in some cases. Antigen dilution titers did not exceed 1:1,000. Antigen dilution titers tended to reach a maximum after twelve injections of sheep serum. In contrast, antiserum dilution titers continued to rise and constant antiserum optional ratios continued to fail as immunization proceeded.

The fallacy of using antigen dilution titer as a measure of the precipitin content of an antiserum is emphasized.

Effect of Diet on Anaphylaxis in the White Rat. Weld, J. T., and Mitchell, L. C.: Proc. Soc. Exper. Biol. & Med. **47**: 168, 1941.

In view of contradictory results reported in studies on anaphylaxis in the white rat the authors investigated the influence of dietary deficiencies in the production of anaphylaxis in this animal.

At weekly intervals the animals were sensitized by three intraperitoneal injections, of either horse serum or sheep serum. The shocking dose was administered intravenously one or two weeks after the last sensitizing injection. The sensitizing and shocking doses were respectively 1 and 1.5 per cent of the animal's body weight.

The addition of yeast, vitamin B, large amounts of casein, and various mineral salts to the usual bread and water diet did not appreciably modify the anaphylactic response in white rats when they were in a fair state of nutrition and were not losing weight. The most important factor in the sensitization of rats was the age of the animal. These animals must be at least 10 weeks old before they can be used successfully for anaphylactic experiments.

Further Observations on Skin Reactions to Antigens From Heterologous Cestodes in Echinococcus Disease. Culbertson, J. T., and Rose, H. M.: J. Clin. Invest. **20**: 249, 1941.

Experiments indicate that antigens capable of eliciting a positive cutaneous reaction in patients with hydatid disease or on cutaneous sites passively sensitized with their serum can be prepared from many different species of cestodes. These include (in addition to the specific larval parasite echinococcus granulosus) *Taenia serrata, T. saginata, T. crassicollis, Hymenolepis fraterna, Moniezia expansa, Raillietina cesticellus,* and *Diphyllobothrium mansonoides.* Suitable antigen occurs in greatest amount in cestodes closely related to the homologous parasite and proportionately less in distantly related forms.

Because of the group character of the reaction to the echinococcus skin test, positive cutaneous reactions obtained with antigens from any of the above cestodes should be viewed critically before being accepted as evidence for infection with echinococcus.

Pediatrics

UNDER THE DIRECTION OF WILLIAM C. DEAMER, M.D., SAN FRANCISCO

The Pathology of Asthma in Children. Traisman, A. S.: Arch. Pediat. **58**: 407, 1941.

This report deals with the pathologic findings in a 1-year-old asthmatic infant. The clinical history was that of a child who had done well until 4 months of age when eczema developed on the face and soon became generalized. Two months later the first attacks of asthma occurred and within two weeks were severe enough to require adrenalin frequently. Skin tests were done and positive reactions were obtained to egg, wheat, milk, beef, barley, apple, carrot, celery, potato, tomato, and to several environmental inhalant substances. All of the foods giving a positive reaction were eliminated excepting milk, which was given in a modified form. Environmental control was instituted. Typical asthmatic breathing was present, and x-ray examination disclosed moderate emphysema in both lower lung fields, as well as a slight increase in the thymic shadow. Two per cent of the white blood cells were eosinophiles.

The infant failed to improve and died in a severe asthmatic attack despite the liberal use of adrenalin. Autopsy revealed a marked emphysema of both lungs, mucous plugs in only two small bronchi, no hypertrophy of the bronchial walls, dilated alveolae, no eosinophiles, small hemorrhages in each lung, perivascular round cell infiltration, and an enlarged thymus gland weighing 23 Gm.

Emotional Factor in Bronchial Asthma in Children. Jensen, R. A., and Stoesser, A. V.: Am. J. Dis. Child. **62**: 80, 1941.

There is increasing recognition being given to the part emotional factors play in asthma, recalling that Osler long classified asthma as a neurosis. Four instances are reported in detail where the emotional factors seemed to play an important role in the production of asthmatic attacks. All were girls between 9 and 16 years of age in an environment with unusual conflicts. Improvement or aggravation of the asthmatic symptoms occurred, dependent upon the emotional status of the individual. None of the four patients cried easily. All had positive skin tests. In each case there had been much discussion of asthma in the child's presence, and an attitude of hopelessness concerning recovery had been permitted to exist.

The authors believe that psychic factors in and of themselves cannot cause asthma, but that they often play a very significant role in the disease.

Skin Testing in the Newborn. B. Zohn, Arch. Pediat. **58**: 339, 1941.

Stimulated by the theory of intrauterine sensitization, the author felt that a reinvestigation of skin reactions in the newborn period was indicated. Balyeat had obtained two markedly positive reactions when 119 newborn infants less than 3 days old were tested to egg, wheat, and milk. Smyth and Bain as well as Weber, Kornfeld, and Walzer had been unable to obtain any positive reactions in the newborn period using the same antigens in equivalent numbers of infants. Zohn tested 150 infants, 3 to 8 days of age, intracutaneously with egg, wheat, and milk extracts, and obtained no positive wheal reactions. Histamine in 1:10,000 dilution consistently caused wheal formation. Twenty-five infants were also tested intracutaneously with wool, feathers, and house dust. Three equivocal wheal reactions were obtained to dust which was recognized as likely to cause nonspecific reactions. Zohn concludes that as far as skin tests are concerned definite evidence of sensitivity to foods at birth is still lacking.

The Management of Infantile Allergic Eczema. Cline, G. M.: Wisconsin M. J. **40**: 789, 1941.

Skin tests are discarded by this author as too uncertain of results to be accepted as a reliable aid in diagnosis in infantile eczema. If some heated form of cow's milk is not satisfactory, goat's milk is tried and, if this is still unsatisfactory, Sobee is given. Should there still be no improvement, the child is put back on heated cow's milk. About half the trials of Sobee have been successful, according to the author. In two instances the too sudden return to the use of cow's milk after a period on soybean milk precipitated edema in the respiratory tract and a severe flare of eczema and resulted in death within thirty-six hours. Toxemia and large areas of skin necrosis were present.

The feeding of cow's milk formulas prior to a breast routine is felt to be undesirable, and dextrose water is suggested instead. Suggestions are also made regarding other foods in the diet and regarding environmental allergens.

The Concept of Atopy in the Light of Recent Investigations. Urbach, E.: Arch. Pediat. **58**: 482, 1941.

The author takes the stand that human allergy and animal anaphylaxis are one and the same thing. He therefore considers Coca's coining of the word "atopy" superfluous. He challenges the points Coca has made differentiating atopy from anaphylaxis and cites experimental and clinical evidence which he feels refutes them. A certain hereditary predisposition to asthma and neurodermatitis is admitted, however, as one of the factors predisposing to allergy.

Reduction of Serum Reactions. Top, F. H., and Watson, E. H.: Am. J. Dis. Child. **62**: 548, 1941.

Using the new "despeciated" type of antitoxin, which has been altered by digestion with taka diastase without impairing its therapeutic value, sixty-one patients with diphtheria and sixty-nine with scarlet fever were given specific antiserum. Reactions were apparently considerably reduced as a result of the alteration of the serum. Six of the sixty-one patients treated with diphtheria antitoxin had some subsequent reaction; two were mild, four moderate, and four severe. This is an incidence of 10 per cent. In a control group of 100 cases receiving ordinary diphtheria antitoxin, 18 per cent had serum sickness which was severe in two cases. Three per cent of the control group were positive by

cutaneous test to horse serum prior to receiving antitoxin, whereas over half of the group receiving despeciated antitoxin had positive tests before serum was given.

An interesting possibility is mentioned that pitressin may help in preventing serum sickness. Of the twelve patients who received it, none developed serum sickness. Eight were in the control group and four in the special group.

Results were quite similar in the sixty-nine scarlet fever patients, where 8 per cent had serum sickness. Fifty-eight per cent of the group had proved positive to horse serum on cutaneous testing.

The expected incidence of serum disease in unselected patients is about 40 per cent according to other authors.

An example of the usefulness of the new serum is the authors' case report of a 13-year-old girl with diphtheria who was extremely sensitive to horse serum. Attempts to desensitize were unsuccessful and resulted in severe reactions. Despeciated antitoxin was well tolerated, without immediate reaction although serum sickness developed on the sixth day.

Rhinology and Ophthalmology

UNDER THE DIRECTION OF FRENCH K. HANSEL, M.D., ST. LOUIS

Sinusitis From the Viewpoint of the General Pathologist. Ash, J. E.: Am. Acad. Ophth. **44**: 304, 1939.

In a study based on the examination of 150 specimens taken from the otolaryngologic registry of the American Academy of Ophthalmology and Otolaryngology at the Army Medical Museum, Washington, D. C., Ash was able to divide the cases of sinusitis represented into the following groups: infectious, seventy-five; allergic, twenty-eight; and mixed, forty-seven. A general summary reveals the scarcity of evidence of any suppurative process in the specimens examined as suggested by the infrequence of polymorphonuclear leucocytes, the prominence in chronic sinusitis of reticulum in the stroma and the basement membrane, the frequent appearance of lymphatic nodes and the development of superficial pseudoglands that may become cysts or abscesses. The last named were observed in thirty-seven specimens. The series shows that hyperplastic changes were observed more frequently than any other pathologic changes in sinusitis of the infectious type and polyps most frequently in cases of the allergic type.

Quantitative Effects of Physical and Chemical Agents on the Erectile Tissue Response in the Nose. Sternstein, H. J.: Arch. Otolaryng. **34**: 523, 1941.

Before investigating the specific action of vasoconstrictor drugs as administered by means of a nasal spray, Sternstein believed that it is essential to evaluate the physiologic effects of certain factors, such as (a) air pressure and (b) the tonicity of a solution, on the erectile tissue response of the inferior turbinate. He found that an air pressure of approximately 15 pounds, commonly employed in nasal sprays, produces little or no erectile tissue response in an unobstructed nose. Air pressure of the same magnitude in an obstructed nose increases both the swelling of the erectile tissue and the degree of nasal obstruction. It is further noted that tonicities of sodium chloride solution of from 0.5 to 5 per cent produced little or no response in an unobstructed nose. In an obstructed nose isotonic and hypotonic solutions gradually increase the swelling of the erectile tissue, producing a corresponding increase of nasal obstruction. A hypertonic solution of 5 per cent decreases swelling of the turbinates and nasal obstruction.

Significance of the pH of Nasal Secretions in Situ. Fabricant, N. D.: Arch. Otolaryng. **34**: 297, 1941.

In his studies of the significance of the pH of nasal secretions in situ, Fabricant suggests that every nasal vasoconstrictor prescribed by physicians and sold to the public should satisfy two basic physiologic requirements: first, it should restore and maintain normal ciliary activity, and second, it should be slightly acid with a pH value in the normal pH range of the nasal secretions in situ, that is, between 5.5 and 6.5. To emphasize the ciliary factor apart from the pH factor is to tell but half the story of applied nasal physiology.

Effect of Silver Preparations and Antiseptics on the pH of Nasal Secretions in Situ. Fabricant, N. D.: Arch. Otolaryng. **34**: 302, 1941.

In a study of the effect of silver preparations and antiseptics on the pH of nasal secretions in situ, Fabricant found that preparations which either enhance or perpetuate the alkalinity of the nasal secretions found in acute rhinitis and acute rhinosinusitis prolong an undesirable nasal status—an alkaline status, in which the bacteria producing the acute inflammation find a fertile field for growth. It is suggested that during these acute processes the employment of a silver preparation which lowers the pH to a level of 5.5 to 6.5 is most desirable. Because of their inherently alkaline composition, metaphen 1:2,500 and 1:500, merthiolate 1:1,000 and 1:5,000, and mercurochrome 0.25 to 2 per cent convert the normal slightly acid nasal status to an alkaline status. According to the observations of Fabricant, this is undesirable. He found that mercresin in a dilution of 1:5 satisfies the pH standards he has advanced by having a pH value that falls within the normal pH range of nasal secretions in situ.

Observations on the Etiology and Treatment of Ménière's Syndrome. Atkinson, M.: J. A. M. A. **116**: 1753, 1941.

Atkinson submits a very comprehensive report of his observations on the etiology and treatment of Ménière's syndrome. He reports two patients in whom foods were the cause of symptoms. Removal of these foods resulted in cessation of the attacks. A group of patients suffering from Ménière's disease were tested with histamine. On the basis of the reaction patients were classified into histamine sensitive and histamine nonsensitive. Seven histamine-sensitive patients were relieved of their attacks following a course of histamine therapy. In two patients a typical attack could be produced by the injection of an adequate amount of histamine.

Book Reviews

Clinical Immunology, Biotherapy and Chemotherapy in the Diagnosis, Prevention, and Treatment of Disease. John A. Kolmer, M.D., and Louis Tuft, M.D., Philadelphia, 1941, W. B. Saunders Co., 941 pages, illustrated.

For those who look upon clinical allergy from the viewpoint of its immunologic aspects as well as for those who have a wider interest in internal medicine, this is a book of first importance. Anaphylaxis and allergy, including bacterial and serum allergy, are integrated with general immunologic concepts. The moot question of the identity of anaphylaxis in lower animals and allergy in man is approached with mature understanding as are other theoretical problems.

Besides the general aspects of immunology, biotherapy, and chemotherapy, most of the book is devoted to the practical applications of the latter two. In so far as allergy is concerned, these include a chapter on the methods of diagnosis and treatment, and another with clinical summaries of asthma, hay fever, and the other common manifestations of allergy. Likewise, the various infectious diseases are taken up in turn and the modern treatment for each discussed.

The clarity and orderliness of presentation, the comprehensiveness of the subject matter and the "up-to-dateness" of factual data identify this book as a model of what a textbook should be. It should be indispensable to internists.

Elimination Diets and the Patient's Allergies; A Handbook of Allergy. Albert H. Rowe, M. D., Philadelphia, 1941, Lea & Febiger, 264 pages.

In the compass of this small book, an extraordinary amount of information is offered. All but sixty pages are devoted to a wide range of topics related to allergy, which in a handbook of this type can only be touched upon. Only about sixty pages deal directly with elimination diets which are well presented and supplemented with directions for their use. There are many practical suggestions and a variety of recipes. New diets have been devised for special purposes.

The italicized point of view that a given elimination diet must be pursued for weeks or months before one can be sure about it, as well as the contention that elimination must be absolute for all patients, will not be shared by all allergists.

From the title of the book, more about elimination diets, on which the author is an authority, was anticipated.

A Manual of Allergy for General Practitioners. Milton B. Cohen, M.D., New York, 1941, Paul B. Hoeber, Inc., 156 pages.

This very small book is scarcely a manual for the general practitioner for he would require more detailed information were he to practice allergy. Nevertheless it is an excellent synopsis of the subject, well arranged and told in understandable style. As a brief introduction to allergy for those who know little about it, this is one of the better books.